Antiretroviral Therapy and Increased Blood Pressure in People Living with HIV in a sub-Saharan African Setting: investigating a plausible causal link using observational data

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Health Sciences (Statistics and Epidemiology)

Warwick Medical School, University of Warwick

September 2016

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To my lovely wife, words cannot describe the immense support you gave to me throughout this process.

ACKNOWLEDGEMENTS

My profound gratitude goes to my supervisors: Dr Olalekan Uthman, Dr Peter Kimani, and Professor Saverio Stranges. Their support and guidance were pivotal to completing this project. The skills I have acquired throughout my doctoral training have been immeasurable because of them: since the commencement of my training, we have published several articles in excellent peer reviewed journals.

My gratitude also goes to Professor Abraham Malu and Dr Godwin Achinge: consultant physicians at the Benue State University Teaching Hospital in Nigeria who provided me with supervision and logistic support during the field work.

I thank the study participants, nursing staff and several ad hoc personnel at the Benue State University Teaching Hospital who were pivotal to conducting the primary study.

I am also thankful to the research degree team at Warwick Medical School. Their support from behind the scenes have not gone unnoticed by me.

To my parents, I am not oblivious to the sacrifices you have made to get me to this point. You have always been my greatest 'cheerleaders' and I love you.

Finally, none of these would have been possible without a fully paid Chancellor's Scholarship from the University of Warwick.

DECLARATION

I hereby declare that this thesis is all my own work except where I have otherwise stated, and that this thesis has not been submitted for a degree at any other university. I also declare that my published work undertaken as part of my doctoral training may form parts of the thesis.

Dr Chidozie U Nduka

September 2016

Who did what?

Evidence synthesis

- Data sources and search strategy: CN, OU
- Inclusion/exclusion criteria: CN, OU
- Quality assessment in each included study: CN, OU
- Data extraction: CN, OU
- Meta-analyses/sensitivity analyses/analyses of publication bias: CN
- Interpretation of the results: CN
- First draft: CN
- Revision of first draft: CN, OU, PK, SS

Mini-review

- Data sources and search strategy: CN, OU
- Inclusion/exclusion criteria: CN, OU
- First draft: CN
- Revision of first draft: CN, OU, PK, SS

Primary data analysis

- Recruited study participants: Study nurses recruited and trained by CN
- Data collection (WHO STEPS instrument, PSQI questionnaire, CES-D questionnaire, SF-12 questionnaire): CN, volunteer research assistants recruited and trained by CN
- Data collection (blood pressure, body mass index and waist circumference measurements): CN, study nurses recruited and trained by CN
- Data collection (capillary blood sampling): laboratory assistants recruited by CN
- Data analyses: CN
- Interpretation of the results: CN
- First draft: CN
- Revision of first draft: CN, OU, PK, SS

CN – Chidozie Nduka; OU – Olalekan Uthman; PK – Peter Kimani; SS – Saverio Stranges; WHO – World Health Organisation; PSQI – Pittsburgh Sleep Quality Index; CES-D – Centre for Epidemiologic Studies Scale; SF-12 – Short Form-12

LIST OF PUBLICATIONS

1. **Chidozie U Nduka**, Ngianga-Bakwin Kandala, Gaurav Suri, Saverio Stranges. Risk factor interventions are effective in preventing cardiovascular events in HIV-infected patients on antiretroviral therapy: A systematic review and meta-analysis. *Circulation* 2013; 127: AP368 (Abstract).
2. **Chidozie U Nduka**, Olalekan A Uthman, Ahmed M Sarki, Saverio Stranges. Impact of antiretroviral therapy on serum lipoprotein levels and dyslipidaemias: A systematic review and meta-analysis. *International Journal of Cardiology* 2015; 199: 307 – 318.
3. **Chidozie U Nduka**, Saverio Stranges, Ahmed M Sarki, Olalekan A Uthman. Evidence of increased blood pressure and hypertension risk among people living with HIV on antiretroviral therapy: a systematic review with meta-analysis. *Journal of Human Hypertension* 2016; 30(6): 355 – 362.
4. Ahmed M Sarki, **Chidozie U Nduka**, Saverio Stranges, Ngianga-Bakwin Kandala, Olalekan A Uthman. Prevalence of hypertension in low- and middle-income countries: a systematic review and meta-analysis. *Medicine* 2015; 94 (50): e1959.
5. **Chidozie U Nduka**, Olalekan A Uthman, Peter K Kimani, Saverio Stranges. Drug abuse in people living with HIV in the era of highly active antiretroviral therapy: a systematic review and meta-analysis. *Journal of Addiction Research and Therapy* 2015; 6: 255.
6. **Chidozie U Nduka**, Olalekan A Uthman, Peter K Kimani, Abraham O Malu, Saverio Stranges. Body fat changes mediate the effects of antiretroviral therapy on blood pressure and blood glucose levels in people living with HIV in a sub-Saharan African setting: a mediation analysis. *Circulation* 2016; 133: AP079 (Abstract).
7. **Chidozie U Nduka**, Saverio Stranges, Peter K Kimani, Olalekan A Uthman. Increased relative weight and body fat distribution in persons living with HIV on antiretroviral therapy: a systematic review and meta-analysis. *AIDS Reviews* 2016; 18(4).

8. **Chidozie U Nduka**, Olalekan A Uthman, Peter K Kimani, Abraham O Malu, Saverio Stranges. Impact of body fat changes in mediating the effects of antiretroviral therapy on blood pressure in HIV-infected persons in a sub-Saharan African setting. *Infectious Diseases of Poverty* 2016; 5(1); DOI: 10.1186/s40249-016-0152-7.
9. **Chidozie U Nduka**, Saverio Stranges, Gerald S Bloomfield, Peter K Kimani, Godwin Achinge, Abraham O Malu, Olalekan A Uthman. A plausible causal link between antiretroviral therapy and increased blood pressure in a sub-Saharan African setting: a propensity score-matched analysis. *International Journal of Cardiology* 2016; 220: 400 – 407.
10. **Chidozie U Nduka**, Saverio Stranges, Ahmed M Sarki, Peter K Kimani, Olalekan A Uthman. Is there sufficient evidence for routine diabetes and metabolic syndrome screening in HIV-infected patients on antiretroviral therapy? A meta-analysis. *Diabetes/Metabolism Research and Reviews* (revision under review).

ABBREVIATIONS

AIDS – Acquired Immune Deficiency Syndrome

ANOVA – One-way Analysis of Variance

ATE – Average Treatment Effect

ATT – Average Treatment Effect on the Treated

CD4 – Cluster of Differentiation 4

CES-D – Centre for Epidemiologic Studies Depression scale

CI – Confidence Interval

HAART – Highly Active Antiretroviral Therapy

HIV – Human Immunodeficiency Virus

HRQL – Health-related Quality of Life

IRIS – Immune Reconstitution Inflammatory Syndrome

MD – Mean Difference

OR – Odds Ratio

PI – Protease Inhibitor

PSQI – Pittsburgh Sleep Quality Index

ROC – Receiver Operating Characteristics

SEM – Structural Equation Modelling

UNAIDS – Joint United Nations programme on HIV/AIDS

ABSTRACT

Background

Whether the epidemiological association between antiretroviral therapy and increased blood pressure is of a causal nature remains largely unknown. The transition from association to causation could represent a fundamental step for taking preventive measures against hypertension and its untoward effects. Such preventive measures are especially crucial for people living with HIV in sub-Saharan African countries, where HIV is most widespread, trends in cardiovascular disease risk factors are rising persistently, and antiretroviral treatment coverage rates are rapidly increasing.

Aims and objectives

- To review the evidence for the epidemiological associations of antiretroviral therapy with increased blood pressure and other cardiovascular disease risk factors, and to obtain comprehensive estimates of the strengths of these associations in people living with HIV worldwide (Evidence synthesis).
- To examine the epidemiological association between antiretroviral therapy and increased blood pressure using Hill's criteria of causation (mini-review).
- To investigate a plausible causal link between antiretroviral therapy and increased blood pressure among HIV-infected patients in a sub-Saharan setting using appropriate statistical methods (primary data analysis).

Methods

Evidence synthesis: A series of systematic reviews with meta-analyses were conducted to examine the associations of antiretroviral therapy with cardiovascular disease risk factors. Studies were sought from electronic databases and cross-references of relevant articles. Studies in which blood pressure and other cardio-metabolic outcomes were compared between HIV-infected adults naïve and exposed to antiretroviral therapy were eligible for the meta-analyses. The data from these studies were combined using random-effects meta-analyses to obtain pooled estimates of the associations between antiretroviral therapy and each cardio-metabolic outcome.

Mini-review: Each of Hill's criteria of causation was examined separately with regards to the association between antiretroviral therapy and increased blood pressure.

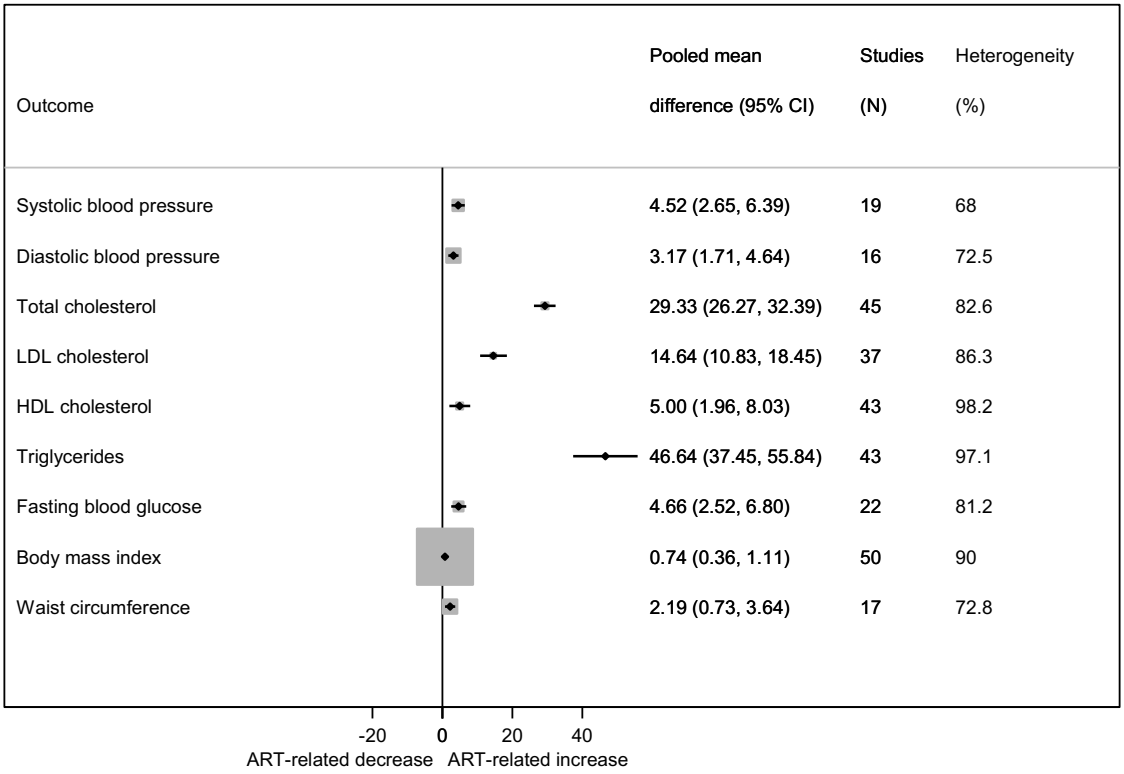
Primary data analysis: Four hundred and six HIV-infected adults—306 antiretroviral-exposed and 100 antiretroviral-naïve—were recruited from a tertiary HIV clinic in semi-urban Nigeria between August and November 2014 as part of a cross-sectional study. To assess if antiretroviral

therapy improves the prediction of hypertension, candidate logistic regression models for predicting hypertension were compared using Nagelkerke's R^2 and parameter estimates. Structural equation models were fitted to determine the indirect effects of antiretroviral therapy on blood pressure through body mass index, waist circumference, blood glucose level, and sleep quality, while controlling for socio-demographic and clinical characteristics. A propensity score matching model was fitted to examine the *average treatment effects on the treated* (ATT) of antiretroviral therapy on systolic and diastolic blood pressure.

Results

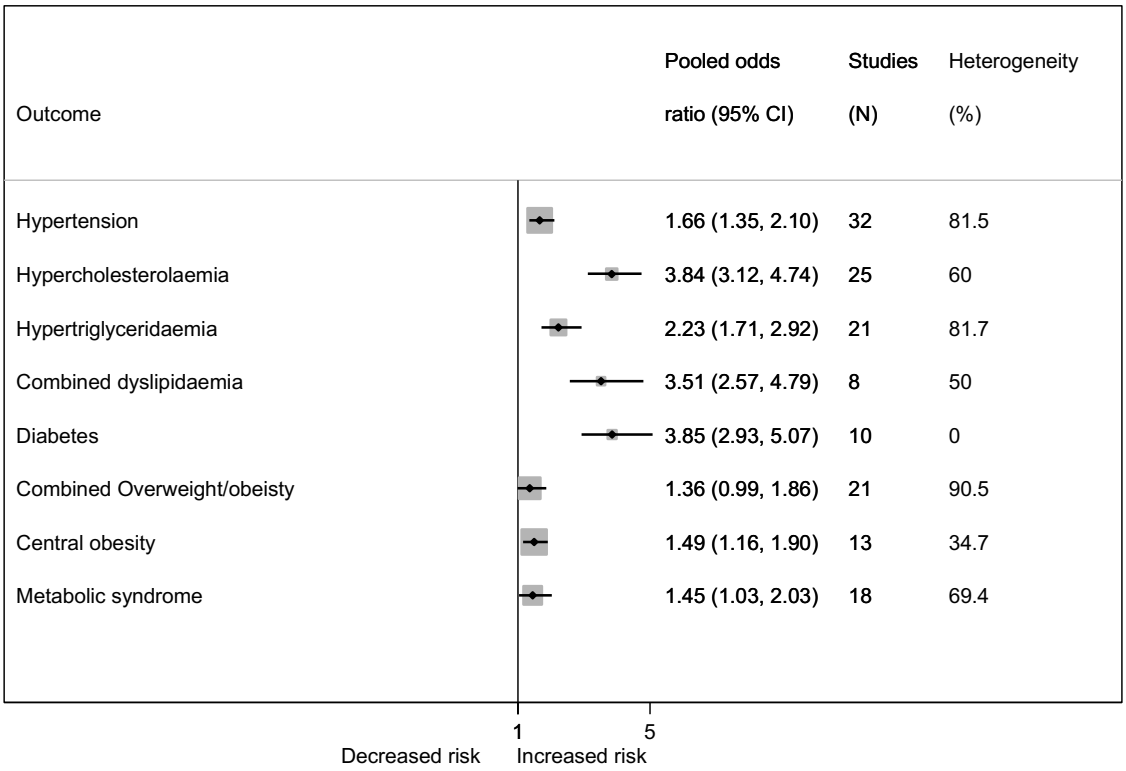
Evidence synthesis: Ninety-one observational studies comprising data for 83,669 HIV-infected patients were included in the meta-analyses. Cardio-metabolic measures were significantly higher among antiretroviral-exposed patients, compared to their naïve counterparts as shown in the forest plots below:

Summary estimates of the pooled associations between antiretroviral therapy and cardio-metabolic outcomes (continuous)



ART, antiretroviral therapy; CI, confidence interval; N, number of studies included in the meta-analyses. Summary plot shows ART is significantly associated with increases in all cardio-metabolic outcome measures.

Summary estimates of the pooled associations between antiretroviral therapy and cardio-metabolic outcomes (categorical)



CI, confidence interval; N, number of studies included in the meta-analyses. With the exception of combined overweight/obesity, ART is significantly associated with increased risk of cardio-metabolic disorders.

Mini-review: The association between antiretroviral therapy and increased blood pressure fulfilled all of Hill's criteria of causation, with the exception of '*specificity*'. Nonetheless, one would not expect the exposure to be specific in the context of a multifactorial condition, such as high blood pressure.

Primary data analysis: Of 400 HIV-infected patients with reported hypertension status, 60 (15%) were diagnosed as hypertensive. Other important findings of primary data analysis include the following:

- Without considering antiretroviral treatment status, the best parsimonious model for predicting hypertension comprised age, body mass index, family history of hypertension, and sleep quality ($R^2 = 0.265$). The extent to which these variables fit within the predictive model was improved slightly when antiretroviral treatment status was included ($R^2 = 0.274$).
- The indirect effects of antiretroviral therapy on systolic (coefficient = 0.94, 95% CI = 0.73 to 2.43) and diastolic blood pressure (coefficient = 0.64, 95% CI = 0.16 to 2.09) through waist circumference remained statistically significant after adjusting for age, sex, lifestyle factors, CD4 cell count, and HIV infection duration.
- After propensity score-matching, 25% of the initial population sample were dropped, so that 229

patients on antiretroviral therapy were matched to 74 antiretroviral-naïve patients. In this propensity score-matched sample, the estimated ATT of the effects of antiretroviral therapy on systolic (7.85 mmHg, 95% CI = 3.72 to 15.68) and diastolic blood pressure (7.45 mmHg, 95% CI = 4.99 to 13.61) remained statistically significant after achieving a balanced distribution of baseline covariates between antiretroviral-naïve and exposed patients.

Conclusion

Overall, antiretroviral therapy is potentially the single most consistent correlate of high blood pressure (and other cardiovascular disease risk factors) in people living with HIV. These findings also suggest a high probability that the epidemiological association between antiretroviral therapy and increased blood pressure may be causal. People living with HIV in sub-Saharan African countries may benefit from regular hypertension screening and other cardiovascular risk assessments after the commencement of antiretroviral therapy. Future studies should identify what phenotypes on the HIV clinical spectrum are most susceptible to the effects of antiretroviral therapy on blood pressure and other cardio-metabolic parameters, as well as the efficacies of targeted interventions on these phenotypes.

PART A

INTRODUCTION

CHAPTER ONE

INTRODUCTION

In this chapter, I briefly set the stage for the thesis, highlighted my personal and professional motivations for my research interest, and presented a brief synopsis of the thesis structure.

1.1 INTRODUCTION

HIV-infected persons now live relatively longer since the advent of highly active antiretroviral therapy (HAART) (Joint United Nations Program on HIV/AIDS [UNAIDS], 2014a; Sabin, 2013; Stanley & Grinspoon, 2012). However, morbidity and mortality rates within this high-risk population remain higher than those observed in the general population (Sabin, 2013). The increasing occurrence of non-AIDS-defining illnesses, such as cardiovascular diseases and cardiovascular disease risk factors, has been identified as one of the underlying factors that may account for this observation (Dau & Holodniy, 2008; Hooshyar *et al*., 2007; Sabin, 2013; Stanley & Grinspoon, 2012). Importantly, the increased life-expectancy among people living with HIV may be associated with substantial blood pressure changes, which are essentially attributable to the direct endothelial-damaging effects of antiretroviral drugs (Seaberg *et al*., 2005; Stein, 2003; Thiebaut *et al*., 2005). Given that more than 35 million people — mostly young and middle-aged adults — live with HIV worldwide (UNAIDS, 2014a), concerns that even small antiretroviral-associated changes in blood pressure may portend considerable public health impact on the incidence and prevalence of hypertension and its untoward effects may be valid, especially as antiretroviral treatment coverage rates continue to

increase globally (UNAIDS, 2014a; UNAIDS, 2014b; World Health Organisation, 2015a).

The associated cardiovascular effects of antiretroviral therapy may potentially constitute a substantial public health burden for the 25 million people who currently live with HIV in sub-Saharan Africa (Mills *et al*, 2011; UNAIDS, 2014a), where antiretroviral treatment coverage rates continue to increase to meet the overwhelming demands associated with increasing HIV prevalence, and where the burden of traditional cardiovascular disease risk factors in people living with HIV continues to grow in the context of an ongoing demographic and epidemiological transition (Bloomfield & Velazquez, 2013; Bloomfield, Hogan & Keter, 2011; Julius, Basu & Ricci, 2011).

The overarching purpose of this study is to examine the epidemiological nature of the association between antiretroviral therapy and cardiovascular disease risk factors, with particular emphasis on investigating the possibility of a causal link between antiretroviral therapy and increased blood pressure in persons living with HIV in semi-urban Nigeria. High blood pressure is widely acknowledged as the most common cardiovascular disorder and the leading risk factor for all-cause mortality (Mathers, Stevens & Mascarehas, 2009; World Heart Federation, 2015; World Health Organization, 2015b). Owing to several factors such as the ongoing nutritional transition, increasing trends in sedentary lifestyle, and other modifiable risk factors, and inadequate health care systems, populations in low- and middle-income countries may bear a higher burden of the disease, compared with the global average (Sarki *et al*., 2015). However, the burden of hypertension is more severe among people living with HIV, compared to the general population (Triant *et al*., 2007):

a trend that has largely been attributed to the advent of HAART (Hooshyar et al., 2007; Dau & Holodniy, 2008). Although several studies have examined the association between antiretroviral therapy (or HAART) and blood pressure changes among people living with HIV in sub-Saharan African countries (Ayodele *et al*., 2013; Dimodi *et al*., 2014; Ekali *et al*., 2013; Manuthu *et al*., 2008; Muhammad, Sani & Okeahialam, 2013a; Muhammad, Sani & Okeahialam, 2013b; Ngala & Fianko, 2013; Ogundahunsi *et al*., 2008), the epidemiological mechanisms involved are less clear. In order to guide the prevention of hypertension and its complications in people living with HIV on antiretroviral therapy in these countries, the causal pathways between antiretroviral therapy and blood pressure changes need to be understood. To the best of the author's knowledge, this study is the first of its kind to determine whether there is a high probability that the epidemiological association between antiretroviral therapy and increased blood pressure is causal.

1.2 PERSONAL AND PROFESSIONAL MOTIVATIONS FOR THE STUDY

My commitment to research in chronic non-communicable disease epidemiology in people living with HIV in low- and middle-income countries informs the topic of this dissertation, and derives from my education and the appropriate field experiences. After my medical training (MBBS) and a short time practicing as a primary care physician in Nigeria, I studied for a Master's in Public Health (MPH) degree at the University of Warwick, achieving a distinction. I went on to work as an HIV/AIDS consultant on a Centre for Disease Prevention and Control/United States Agency for International Development (CDC/USAID) funded project in Nigeria. I was one of two physicians treating HIV-infected patients in a rural facility with a patient-base of about 5000. I assessed adult and pediatric HIV-infected patients for eligibility to commence

antiretroviral therapy, while monitoring their response to the treatments. I also supervised prevention-of-mother-to-child-transmission (PMTCT) services, which entailed antiretroviral prophylaxis for all pregnant mothers and newborns, as well as preliminary and confirmatory testing for infants born to HIV-infected mothers.

Technically, my introduction to the field of cardiovascular disease epidemiology in people living with HIV began with the Masters in Public Health professional project, which was a systematic review and study-by-study analysis of randomized controlled trials appraising the effectiveness of risk factor interventions for preventing cardiovascular events in people living with HIV on antiretroviral therapy. My research projects have since been focused on cardiovascular disease risk factors in persons living with HIV.

Working with HIV-infected patients in clinical settings in Nigeria and Kenya, I have gained first-hand experience of the devastating effects of HIV infection in these high risk and — and often impoverished — populations. In addition to the social stigma that these patients are often forced to endure in the community, they are marginalised by a health system that knows very little about the potential public health implications of expanding antiretroviral treatment coverage, notably the rising occurrence of cardio-metabolic disorders with their complications. Therefore, a number of cardio-metabolic disorders, such as dyslipidaemias and diabetes mellitus, tend to be underdiagnosed following enrolment into treatment. Even when cardio-metabolic disorders are eventually detected (such as high blood pressure), they (cardio-metabolic disorders) are often treated in isolation from the potential complications of HIV infection and antiretroviral therapy. Based on my clinical experience, benefits derived from such treatments are usually not sustainable long term, most probably because the treatment

choices are not informed by an understanding of the complex epidemiological pathways between antiretroviral therapy and increased blood pressure.

It is clear that the care and treatment of most persons living with HIV in Nigeria (and several other sub-Saharan African countries) do not extend beyond antiretroviral therapy, surveillance of clinical indications for the commencement of antiretroviral therapy, and the treatment of opportunistic infections. Barring the austere political climate and other social factors in the country, it is plausible that the reasons why HIV-infected patients do not generally get the extra care for hypertension and other cardio-metabolic disorders after the commencement of antiretroviral therapy may rely heavily on the dearth of evidence — especially data originating from the sub-Saharan African region — uncovering the complex nature of the epidemiological associations between antiretroviral therapy and cardio-metabolic changes. It is my goal that antiretroviral-associated increases in blood pressure and other cardio-metabolic parameters be no longer neglected in sub-Saharan African settings because of a lack of evidence, but be recognised as potential public health problems with dire consequences if left unaddressed. It is also my goal that country-specific guidelines for the care and treatment of people living with HIV across the sub-Saharan African region entail comprehensive baseline and routine cardiovascular risk assessments. Drawing on all of the above, I sought to answer the research questions posed in the thesis.

1.3 A BRIEF SYNOPSIS OF THE THESIS STRUCTURE

PART A: Introduction (Chapters 1 to 3)

In this section, I set the stage for my doctoral dissertation, while highlighting the motivation and theoretical basis for conducting the research. The scientific context for

the thesis is explained comprehensively, including an account of the epidemiology of cardiovascular disease risk factors in persons living with HIV in the era of highly active antiretroviral therapy, current treatment strategies for cardio-metabolic disorders in persons living with HIV on antiretroviral treatment, the potential consequences of antiretroviral-associated cardio-metabolic disorders from a public health perspective, and the underlying issues that may perpetuate the potential double burden of HIV and antiretroviral-associated cardio-metabolic disorders in sub-Saharan African countries, where health indices are worse off. The research questions, overall aims and objectives of the dissertation are outlined in this section. The study hypotheses and conceptual framework are also illustrated.

***PART B*: Global evidence synthesis of the association between antiretroviral therapy and cardiovascular disease risk factors (Chapters 4 to 11).**

The evidence for the associations of antiretroviral therapy with increased blood pressure and other cardiovascular disease risk factors in people living with HIV are reviewed. Meta-analyses of the pooled estimates of these associations are also presented. Background, methods and results chapters are presented in this section.

***PART C*: An Epidemiological Examination of the Association between Antiretroviral Therapy and Blood Pressure Changes using Hill's Criteria of Causation (mini-review) (Chapters 12 to 14).**

Each of Hill's criteria of causation is examined in relation to the association between antiretroviral therapy and increased blood pressure. Background, methods and results chapters are also presented in this section.

***PART D*: Primary data collection and analysis in a resource-limited setting (Chapters 15 to 21).**

In this section, alternative causal pathways between antiretroviral therapy and increased blood pressure among people living with HIV in semi-urban Nigeria are examined based on appropriate statistical analyses of primary observational data collected by me. The activities of this section also entail a background, methods and results chapters.

***PART E*: Discussions of the findings and recommendations (Chapters 22 to 26).**

In this section, I interpret the results of the dissertation, highlighting its practical and research implications, limitations and strengths. I also the findings of the dissertation, while reinforcing the main ideas.

CHAPTER TWO

BACKGROUND

In this chapter, I describe the scientific context of the thesis, including a succinct description of the problem, certain epidemiological facts about the problem, current treatment strategies, consequences / associated burden, and any underlying social issues that may perpetuate the problem in sub-Saharan African settings, where health indices tend to be worse off.

2.1 ANTIRETROVIRAL THERAPY AND CARDIOVASCULAR DISEASE RISK FACTORS

Deaths arising from the Acquired Immune Deficiency Syndrome (AIDS) have declined substantially since the advent of highly active antiretroviral therapy (HAART) (Joint United Nations Program on HIV/AIDS [UNAIDS], 2014a; Van Vugt *et al*., 2007). HAART entails the treatment of HIV infection using two or more drugs from different antiretroviral drug classes, notably the protease inhibitors and non-nucleoside reverse transcriptase inhibitors. Other benefits of HAART include suppression of viral load, improvements in cell-mediated immunity, and decreased occurrence of opportunistic infections (Dagogo-Jack, 2008). However, the improved life-expectancy among persons living with HIV may be associated with a high occurrence of cardiovascular disease risk factors, such as high blood pressure (Seaberg *et al*., 2005; Thiébaut *et al.,* 2005), raised serum lipid levels (Dubé *et al*., 2003; Shlay *et al*., 2007), high blood glucose concentrations (Dagogo-Jack, 2008; Kalra *et al*., 2011), body fat changes (Grunfeld *et al*., 2010; Shlay *et al*., 2007), and metabolic syndrome (Krishnan *et al*., 2012). Of note, HAART exacerbates the severity and prevalence of these non-AIDS-defining illnesses

(Dillon *et al*., 2013). However, it is difficult to differentiate the clinical presentations of antiretroviral-associated cardio-metabolic disorders from cardio-metabolic conditions of other origins — a problem which potentially complicates the clinical diagnoses and subsequent treatment of cardiovascular conditions in HIV-infected patients receiving antiretroviral therapy (Yarasheski *et al*. 1999; Dagogo-Jack, 2008).

2.2 EPIDEMIOLOGICAL TRENDS

2.2.1 Mechanisms of cardio-metabolic changes in people living with HIV

Antiretroviral therapy underlies the pathogenesis of cardio-metabolic disorders in a substantial proportion of people living with HIV, and this through different mechanisms that may not be linked to specific classes of antiretroviral drugs (Kalra *et al*. 2011). For instance, both reverse transcriptase and protease inhibitors directly damage the endothelial linings of blood vessels, subsequently inhibiting the production of biological markers that help to regulate blood pressure, such as nitric oxide (Seaberg *et al*., 2005; Stein, 2003). Regardless of the class to which antiretroviral drugs belong, they also cause blood pressure changes by increasing large arterial wall stiffness, which has been identified as a causative factor in hypertension (Ngatchou *et al*., 2013; Wagenseil & Mecham, 2012). Both nucleoside reverse transcriptase inhibitors and protease inhibitors alter lipid metabolism by inhibiting mitochondrial function, which in turn, increases biosynthesis and reduces hepatic clearance of low density lipoprotein (LDL) cholesterol (Fleischman *et al*., 2007; Stein, 2003). Although the mechanisms of antiretroviral-induced changes in serum lipoprotein levels may overlap between different antiretroviral drug classes, the degree to which serum lipoproteins are altered may differ between antiretroviral drugs and antiretroviral classes. For instance, total cholesterol and LDL cholesterol levels are increased more markedly following treatment using efavirenz,

lopinavir and ritonavir, as opposed to atazanavir and darunavir (Daar *et al*., 2011; Molina *et al*, 2010). Indinavir interferes with glucose metabolism by inducing insulin resistance without any indirect effects on lipid metabolism, whereas lopinavir and ritonavir alter lipid metabolism without mediating insulin resistance (Kalra *et al*., 2013; Woerle *et al*., 2003). Protease inhibitors and nucleoside reverse transcriptase inhibitors also inhibit glucose metabolism by mediating mitochondrial dysfunction (Dagogo-Jack, 2008; Kalra *et al*., 2013). Occasionally, antiretroviral treatment using either protease inhibitors or reverse transcriptase inhibitors may overstimulate the immune system, consequently increasing the production of autoantibodies that target the enzyme: glutamic acid decarboxylase (GAD) in persons who would later develop type 1 (or autoimmune) diabetes mellitus (Takarabe *et al*., 2010). Both isoforms of glutamic acid decarboxylase (GAD_{67} and GAD_{65}) are present in the pancreas, and are responsible for the synthesis of gamma aminobutyric acid (GABA), which is an important neurotransmitter in the pancreatic islet cells (Gilliam, Palmer & Lernmark, 2004). Hence, an autoimmune response against GAD in the pancreatic cells interferes with insulin production, leading to type 1 diabetes (Baekkeskov *et al*., 1990; Kaufman *et al*., 1992). Although the underlying mechanisms explaining the association between antiretroviral therapy and body fat changes are unclear, evidence suggests a plausible link between antiretroviral drugs and raised serum cortisol levels, suggestive of Cushing's disease (Stanley & Grinspoon, 2012): a metabolic disorder that invariably presents with central adiposity and relative weight gain. In spite of the similarities in the mechanisms by which protease inhibitors and reverse transcriptase inhibitors may increase cardiovascular risk in persons living with HIV, evidence in the available literature allude to differential cardio-metabolic effects between both classes of antiretroviral drugs, with protease inhibitors associated with a more acute symptomatology than reverse transcriptase inhibitors (Kalra *et al*.,

2013; Shlay *et al*., 2007). While there are newer classes of antiretroviral drugs — such as the integrase inhibitors, fusion inhibitors, and chemokine receptor antagonists — that are less likely to be associated with adverse cardio-metabolic effects (Rathbun, Liedtke & Lockhart, 2013), it is worth emphasizing that these newer drugs are not widely available because they have not been approved for use in most countries (Deeks *et al*., 2008; Rathbun, Liedtke & Lockhart, 2013). In fact, the newer classes of antiretroviral drugs have not been rolled out in any low- or middle-income country.

HIV infection, in itself, may aggravate the effects of antiretroviral drugs on cardiovascular disease risk factors, through its chronic inflammatory and platelet activating effects (Dau & Holodniy, 2008; Mutimura *et al*., 2008). Although these mechanisms have not been fully elucidated, a recurring hypothesis revolves around a relationship between high viral loads and increased inflammation (Strategies for Management of Antiretroviral Therapy [SMART] Study Group, 2006).

2.2.2 Blood pressure changes / hypertension in people living with HIV

According to the World Heart Federation (2015b), at least 970 million people of the general population have hypertension, with the largest prevalence in the African continent. Although the worldwide burden of hypertension in high-risk populations such as people living HIV remains largely unknown, it is considered to be more severe when compared with the general population. A secondary analysis of data on a large number of patients stratified by HIV status revealed a significantly higher prevalence of hypertension among HIV-seropositive patients, compared to seronegative patients (21.2% *versus* 15.9%; $P < 0.001$) (Triant *et al*., 2007). Kaplan *et al*. (2007) found the age-adjusted prevalence of hypertension to be 24% in a cohort of 2386 HIV-infected subjects.

However, the higher prevalence of hypertension in HIV-infected persons, compared to the general population, may largely be attributable to the effects of antiretroviral therapy. For instance, findings from the Multicentre AIDS Cohort Study (MACS) revealed that the risk of hypertension increased 1.5 fold in HIV-infected men following the commencement of HAART (Seaberg *et al*., 2005). Chow *et al*. (2003) also found statistically significant increases in systolic and diastolic blood pressure for every additional year of HAART, even after adjusting for age, blood pressure at baseline and CD4 cell count. HIV-infected patients exposed to HAART may be up to ten times more likely to be hypertensive, compared with HIV-infected patients who are naïve to antiretroviral treatment (Ekali *et al*., 2013).

2.2.3 Blood glucose changes / diabetes mellitus in people living with HIV

Triant *et al*. (2007) also found a significantly higher prevalence of Type 2 diabetes mellitus in HIV-infected patients, compared to those who were HIV-negative (11.5% *versus* 6.6%; $P < 0.001$). Brown *et al*. (2005) also found HIV-infected patients to be approximately four times more likely to develop Type 2 diabetes, compared with HIV-negative individuals. Yarasheski *et al*. (1999) suggested that genetic predisposition was the single most consistent predictor of diabetes among people living with HIV; however, this assertion was made early on in the HAART era, so that there was a dearth of studies reporting abnormalities of glucose metabolism associated with antiretroviral therapy at the time. A literature search did not find studies published prior to the year 2000 that had examined the associations between antiretroviral therapy and diabetes mellitus risk. Antiretroviral therapy has been identified as a major predictor of diabetes mellitus in people living with HIV (Larsson et *al*., 2006): about one in ten HIV-infected patients on antiretroviral therapy may present with diabetes mellitus (Calza *et al*., 2011; Kalra & Agrawal, 2013). HIV-infected patients are also likely to develop diabetes mellitus as a

result of the increased body weight that often follows antiretroviral treatment (Dagogo-Jack, 2008; Kalra *et al*., 2011). The clinical presentation of antiretroviral-associated diabetes mellitus is often consistent with type 2 diabetes (Dagogo-Jack, 2008; Kalra *et al*., 2011). However, a few patients may present with a form of type 1 diabetes that is autoimmune-related: the outcome of an exaggerated immune response to antiretroviral treatment (Kalra *et al*., 2011; Takarabe *et al*., 2010).

2.2.4 Serum lipid changes / dyslipidaemias in people living with HIV

Interestingly, evidence from the pre-HAART era reported decreases in serum cholesterol levels and slight increases in serum triglycerides among HIV-infected patients prior to developing AIDS (Grunfeld *et al*., 1991; Grunfeld *et al*., 1992; Shor-Posner *et al*., 1993). Mujawar *et al*. (2006) had suggested that the inhibition of cholesterol efflux from human macrophages by the human immunodeficiency virus was a plausible mechanism explaining why HIV infection causes a shift in serum lipoprotein levels, however, there is no consensus regarding this hypothesis. Regardless of antiretroviral treatment status, previous studies have consistently reported higher prevalence estimates of dyslipidaemia in HIV-infected patients, compared to non-infected patients. For instance, Triant *et al*. (2007) reported a significantly higher prevalence of dyslipidaemia in HIV-infected patients, compared to non-infected patients (23.3% *versus* 17.6%; $P < 0.001$). However, it is clear that antiretroviral therapy exacerbates the abnormalities of lipid metabolism in HIV-infected patients (Carey *et al*., 2010; Daar *et al*., 2011; Dillon *et al*., 2013; Dube & Fenton, 2003; Fleischman *et al*, 2007; Fontas *et al*., 2004; Molina *et al*, 2010; Pujari *et al*., 2005; Riddler *et al*., 2003; Riddler *et al*., 2007; Stein, 2003). For example, in a Multi-center AIDS Cohort Study (MACS), Riddler *et al*. (2003) observed significant declines in mean serum concentrations of total cholesterol, HDL cholesterol, and LDL cholesterol

among HIV-infected men followed-up for a mean duration of eight years, compared to pre-seroconversion concentrations. However, the subsequent initiation of antiretroviral therapy was associated with increases in serum concentrations of total cholesterol and LDL cholesterol, but no significant change in HDL cholesterol levels (Riddler *et al*., 2003). Furthermore, prevalence estimates of dyslipidaemia among people living with HIV on antiretroviral therapy may be as high as 80% — depending on the characteristics of the study population (Troll, 2011).

2.2.5 Body fat changes in people living with HIV

Although antiretroviral-naive HIV-infected patients are more likely to develop body fat changes, compared to non-infected patients, exposure to antiretroviral therapy may exacerbate these changes (Shlay *et al*., 2007). More than one in two HIV-infected patients may develop body fat changes, especially central fat accumulation, soon after commencing HAART (Grunfeld *et al*., 2010; Shlay *et al*., 2007). Evidence from more recent studies suggest that antiretroviral-exposed patients may be up to five times more likely than those naïve to treatment to become overweight or obese (Peck *et al*., 2014), and more than two times more likely to become centrally obese (Blass *et al*., 2008; Muhammad, Sani & Okeahialam, 2013a; Muhammad, Sani & Okeahialam, 2013b).

2.2.6 Metabolic syndrome in people living with HIV

Metabolic syndrome is a constellation of prediabetes, dyslipidaemia, central obesity, and high blood pressure. More recently, there have been other metabolic abnormalities proposed to be included in the definition of metabolic syndrome, including hyperuricaemia (an excess of uric acid in the blood), microalbuminuria (small increases in the albumin levels in urine), hypercoagulability (coagulopathy that increases the risk

of clot formation in blood vessels), and vascular inflammation (vascular condition characterized by formation of atheromatous plaques in arterial walls) (Blaton, Korita & Bulo, 2008). Like its individual components, metabolic syndrome as a pathophysiological entity increases the risk of cardiovascular disease in the general population; however, among persons living with HIV, the presence of metabolic syndrome as an entity is no better than the sum of its individual components in predicting cardiovascular risk (Worm *et al*., 2009). The direct effects of antiretroviral drugs on endothelial and adipocyte function, lipid metabolism, and mitochondrial dysfunction sufficiently explain the link between antiretroviral therapy and metabolic syndrome; however, the role of a genetic predisposition to metabolic syndrome in persons living with HIV on antiretroviral therapy has also been suggested (Babaro & Lacobelli, 2009). The prevalence of metabolic syndrome in people living with HIV on antiretroviral therapy has been shown to vary widely between 18% and 83%, with higher prevalence estimates found among older persons; patients on protease inhibitor-based antiretroviral regimens; and patients exposed to antiretroviral treatment for longer durations (Babaro & Lacobelli, 2009).

2.2.7 The role of lifestyle factors in people living with HIV

Lifestyle factors such as smoking, heavy alcohol drinking and poor dietary habits may also underlie the aetiology of cardio-metabolic disorders among people living with HIV on antiretroviral therapy. Savès *et al*. (2003) reported significantly higher prevalence rates of smoking in HIV-infected men (56.6% *versus* 32.7%; $P < 0.001$) and women (58% *versus* 28%; $P < 0.001$) who received antiretroviral treatment, compared to their respective counterparts in the general population. Smoking also reduces the potency of antiretroviral drugs, causing HIV-infected patients who smoke to become more susceptible to the inflammatory effects of the virus, compared to HIV-infected patients

who do not smoke (Crothers *et al*., 2005; Feldman *et al*., 2006). Similarly, Baum *et al*. (2010) found a correlation between alcohol abuse and increased viral load among HIV-infected patients on antiretroviral therapy (P = 0.046), which may explain by the connection between alcohol abuse and sub-optimal antiretroviral adherence (Samet *et al*., 2004). In other words, the role of alcohol abuse in increasing the risk of cardio-metabolic disorders is somewhat coherent with evidence from the SMART trial suggesting a relationship between high viral loads and increased inflammation (SMART Study Group, 2006). Increased dietary fat intake — found to be more prevalent in HIV-infected patients compared to the general population — has also been identified as a significant contributor to the high prevalence of dyslipidaemia and central obesity among HIV-infected patients in the HAART era (Jaime *et al*., 2006; Joy *et al*., 2007).

2.2.8 The role of socioeconomic factors in people living with HIV

Socioeconomic factors may also play an important role in the epidemiology of hypertension and other cardiovascular disease risk factors in people living with HIV. For instance, as observed in the general population, persons living with HIV in socially deprived settings may be more likely to be at risk of cardiovascular diseases, compared to HIV-infected persons who live in less deprived areas (Marmot *et al*., 1997; Wilkinson & Marmot, 2003). Wester *et al*. (2011) further supports this claim by showing higher incidence rates of cardiovascular diseases among antiretroviral-exposed HIV-infected patients living in Botswana, compared with those in the United States (8.4 *versus* 5.0 per 1,000 person-years). It is also plausible that the link between socioeconomic status and lifestyle, as observed in landmark studies investigating the socioeconomic and psychosocial determinants of health inequalities (Hemingway & Marmot, 1999; Marmot *et al*., 1997; Wilkinson & Marmot, 2003), may partially account for this phenomenon.

For instance, Batista *et al*. (2013) found that HIV-infected patients who were lower income earners and without any formal education were more likely to be current smokers, compared to their counterparts who were higher income earners and educated.

2.2.9 The role of non-traditional risk factors in people living with HIV

Other factors such as sleep problems (Hoevenaar-Blom *et al*., 2011; Stranges *et al*., 2008), depressive symptoms (Joynt, Whellan & O'Connor, 2003; Wassertheil-Smoller *et al*., 2004), and impaired health-related quality of life (Stranges *et al*., 2015) which are known to be associated with increased risk of hypertension and other cardio-metabolic disorders in the general population, have not been sufficiently explored in relation to cardio-metabolic health in people living with HIV. Nonetheless, these factors may potentially contribute to the aetiology of cardio-metabolic disorders in HIV-infected persons on antiretroviral therapy, given their high occurrence in this high-risk subgroup (Wibbeler *et al*., 2012). For instance, approximately 59% of HIV-infected patients in a cross-sectional study were assessed to have poor sleep quality, the odds of which were more than four times higher among patients exposed to HAART, compared to HAART-naïve patients (Oshinaike *et al*., 2014). Similarly, prevalence estimates of depression, which is considered the most common mood disorder among people living with HIV, may be as high as 50% in people living with HIV on antiretroviral therapy (Hartzell, Janke & Weintrob, 2008). Although there is a lack of data for comparisons of mood disorders between HAART-exposed and HAART-naïve patients, HAART-exposed patients may be up to four times more likely to have mood disorders, compared with the general population (Pence *et al*., 2006).

2.3 CURRENT TREATMENT STRATEGIES

In spite of the accompanying adverse effects, withholding antiretroviral treatment cannot be an option, given that most people living with HIV who are eligible to commence HAART succumb to AIDS within two to three years without treatment (Dau & Holodniy, 2008). Antiretroviral non-adherence may also increase cardiovascular risk through mechanisms earlier described: the SMART Study Group (2006) exemplifies this dilemma, having found a higher risk of cardiovascular disease associated with interrupting antiretroviral treatment (Hazard Ratio = 1.6, 95% CI = 1.0 to 2.5, $P = 0.05$). Evidence from randomized clinical trials suggest that therapeutic lifestyle interventions, including dietary modification and increased physical activity are effective in preventing cardio-metabolic disorders in HIV-infected patients on HAART (Balasubramanyam *et al*., 2011; Baril *et al*., 2007; Fitch *et al*., 2006). Interventions such as anti-hypertensive drugs, lipid lowering medications, and oral hypoglycaemic agents have also been effective in treating HAART-exposed patients presenting with hypertension, dyslipidaemia, and type 2 diabetes mellitus respectively (Calza, Manfredi & Chiodo, 2003; Calza *et al*., 2005). However, these pharmacological agents may be more effective than standard treatment when they are intensified to target lower reference values for blood pressure, serum lipoprotein levels, and other cardiovascular risk parameters (Masia *et al*., 2009). Furthermore, antiretroviral switch therapy, where antiretroviral drugs associated with adverse cardio-metabolic effects are replaced with those that have better side-effect profiles, may also be more effective than standard treatment in managing antiretroviral-associated cardio-metabolic disorders (Masia *et al*., 2009; Rasmussen *et al*., 2011). The second generation protease inhibitors (e.g. atazanavir, darunavir), as well as the newer classes of antiretroviral drugs (e.g. integrase inhibitors, fusion inhibitors and chemokine receptor antagonists) may represent the drugs of choice for treating

antiretroviral-associated cardio-metabolic disorders in people living with HIV (Deeks *et al*., 2008; Rathbun, Liedtke & Lockhart, 2013).

2.4 CONSEQUENCES (ASSOCIATED BURDEN)

Overall, previous studies suggest that about 60% of persons living with HIV on antiretroviral therapy may develop one or more risk factors for cardiovascular diseases (Carr *et al*., 1998; Dube & Sattler, 1998; SoRelle, 1998; Troll, 2011), approximately half of whom may progress to coronary heart disease, stroke or cardiovascular mortality (Friis-Møller *et al*., 2003). Although there is a dearth of studies reporting quality and disability-adjusted life-years associated with antiretroviral-associated cardiovascular disease risk factors, HIV and cardiovascular disease remain two major causes of morbidity and mortality worldwide, and potentially constitute a double burden, especially in sub-Saharan African countries (Bloomfield & Velazquez, 2013). Projections anticipate that HIV and cardiovascular disease could account for the majority of global morbidity and mortality by the year 2030, which would remain largely driven by morbidity and mortality rates in low- and middle-income countries (Mathers & Loncar, 2005; Kearney *et al*., 2005). Furthermore, the UNAIDS's (2014a) commitment to end the AIDS epidemic by 2030 through strategic scale-up of antiretroviral treatment services may portend a substantial public health burden with respect to a potential growth to epidemic proportions of antiretroviral-associated cardio-metabolic disorders, and potentially worsening the already-existing global burden of hypertension, diabetes, and other cardio-metabolic disorders with their complications. With approximately 36 million people currently living with HIV worldwide, 13 million people with access to antiretroviral treatment, and a projected 18 million incident cases by 2030 (UNAIDS 2014b), concerns that antiretroviral-associated cardio-metabolic disorders may become epidemic are valid.

2.5 THE SUB-SAHARAN AFRICAN CONTEXT

At least 25 million people are living with HIV across the sub-Saharan African region, which accounts for more than 70% of the global population of HIV-infected persons. In the past decade, there have been at least 1.3 million new HIV infections across sub-Saharan African countries every year, which also accounts for approximately 70% of the global incidence of HIV infection. Although AIDS-specific mortality rates have fallen by 27% since the advent of HAART in sub-Saharan Africa in 2001, the region still accounts for more than 70% of the global total of AIDS-related deaths. Two out of three HIV-infected patients across sub-Saharan African countries who are eligible to commence HAART do not have access to treatment, even though 75% of the global total of people living with HIV on antiretroviral therapy reside in these countries, and recent trends confirm sustained increases in antiretroviral coverage rates since the introduction of HAART in the region (UNAIDS 2013; UNAIDS 2014b; World Health Organization, 2015a).

Unlike the developed world, there are hardly any surveillance systems in place for monitoring cardio-metabolic disorders in people living with HIV in sub-Saharan African countries. Care and treatment guidelines for HIV-infected patients in most of these countries do not entail baseline or routine cardiovascular risk assessments. In addition, the governments of these countries, as well as the various international donor agencies, do not usually subsidise investigations required for comprehensive cardiovascular risk assessments (Bloomfield & Velazquez, 2013), potentially constituting significant direct costs to the patients, most of whom are socio-economically deprived and required to pay out-of-pocket for health care.

While antiretroviral switch therapies are widely used to treat HAART-associated cardio-metabolic disorders in developed countries (Calza *et al*., 2005; Masia *et al*., 2009; Rasmussen *et al*., 2011), such treatment options are not readily available in sub-Saharan African countries. The non-nucleoside reverse transcriptase inhibitor-based HAART regimens are used as first line treatment for HIV infection in sub-Saharan African countries, whereas the protease inhibitor-based HAART regimens are used in cases of first line treatment failure (Federal Ministry of Health Nigeria, 2012; Ministry of Medical Services Kenya, 2011; UNAIDS & World Health Organisation, 2006). The more expensive newer classes of antiretroviral drugs (such as the integrase inhibitors, fusion inhibitors, and chemokine receptor antagonists) have yet to be approved for use in sub-Saharan African countries, potentially resulting in an overreliance on antihypertensive, hypoglycaemic and lipid lowering medications, which are modestly effective for modifying cardio-metabolic risk (Glass *et al*., 2006; Nuesch *et al*., 2013), and potentially interact with antiretroviral drugs (Dau & Holodniy, 2008).

CHAPTER THREE

RESEARCH QUESTIONS, AIMS AND OBJECTIVES, AND HYPOTHESES

In this chapter, I describe the research questions, the overall aims and objectives, and hypotheses of the thesis. I also present a conceptual framework upon which the hypotheses were based.

3.1 RESEARCH QUESTIONS

I. What is the impact of antiretroviral therapy on cardiovascular disease risk factors in people living with HIV?

II. Is there a plausible causal link in the epidemiological association between antiretroviral therapy and increased blood pressure?

3.2 AIMS AND OBJECTIVES

3.2.1 First aim (Global evidence synthesis)

To examine the impact of antiretroviral therapy on cardiovascular disease risk factors in people living with HIV.

3.2.1.1 Objectives

I. To conduct a series of systematic reviews and meta-analyses of studies examining the epidemiological associations between antiretroviral therapy and changes in the following cardio-metabolic parameters:

- Blood pressure and hypertension.
- Serum lipoprotein levels and dyslipidaemias.
- Blood glucose levels and diabetes mellitus.
- Body fat measures, combined overweight/obesity, and central obesity.

- Metabolic syndrome.

II. To examine the epidemiological association between antiretroviral therapy and increased blood pressure using Hill's criteria of causation.

3.2.2 Second Aim (Primary data collection and analysis in a resource-limited setting)

To investigate a plausible causal link between antiretroviral therapy and increased blood pressure in a sub-Saharan African setting.

3.2.2.1 Objectives

I. To examine the impact of antiretroviral treatment status on the clinical prediction of hypertension in people living with HIV in a sub-Saharan African setting.

II. To examine alternative causal pathways that potentially mediate the effects of antiretroviral therapy on blood pressure in people living with HIV in a sub-Saharan African setting.

III. To estimate the causal average treatment effect of antiretroviral therapy on blood pressure in people living with HIV in a sub-Saharan African setting.

3.3 HYPOTHESES AND CONCEPTUAL FRAMEWORK

Figure 3.1 illustrates the conceptual framework upon which the research hypotheses are based. The hypotheses outlined below are based on the relationships depicted in the conceptual framework:

I. Antiretroviral therapy is associated with cardiovascular disease risk factors in people living with HIV (not shown in the diagram).

II. Antiretroviral treatment status improves the clinical prediction of hypertension in people living with HIV in a sub-Saharan African setting.

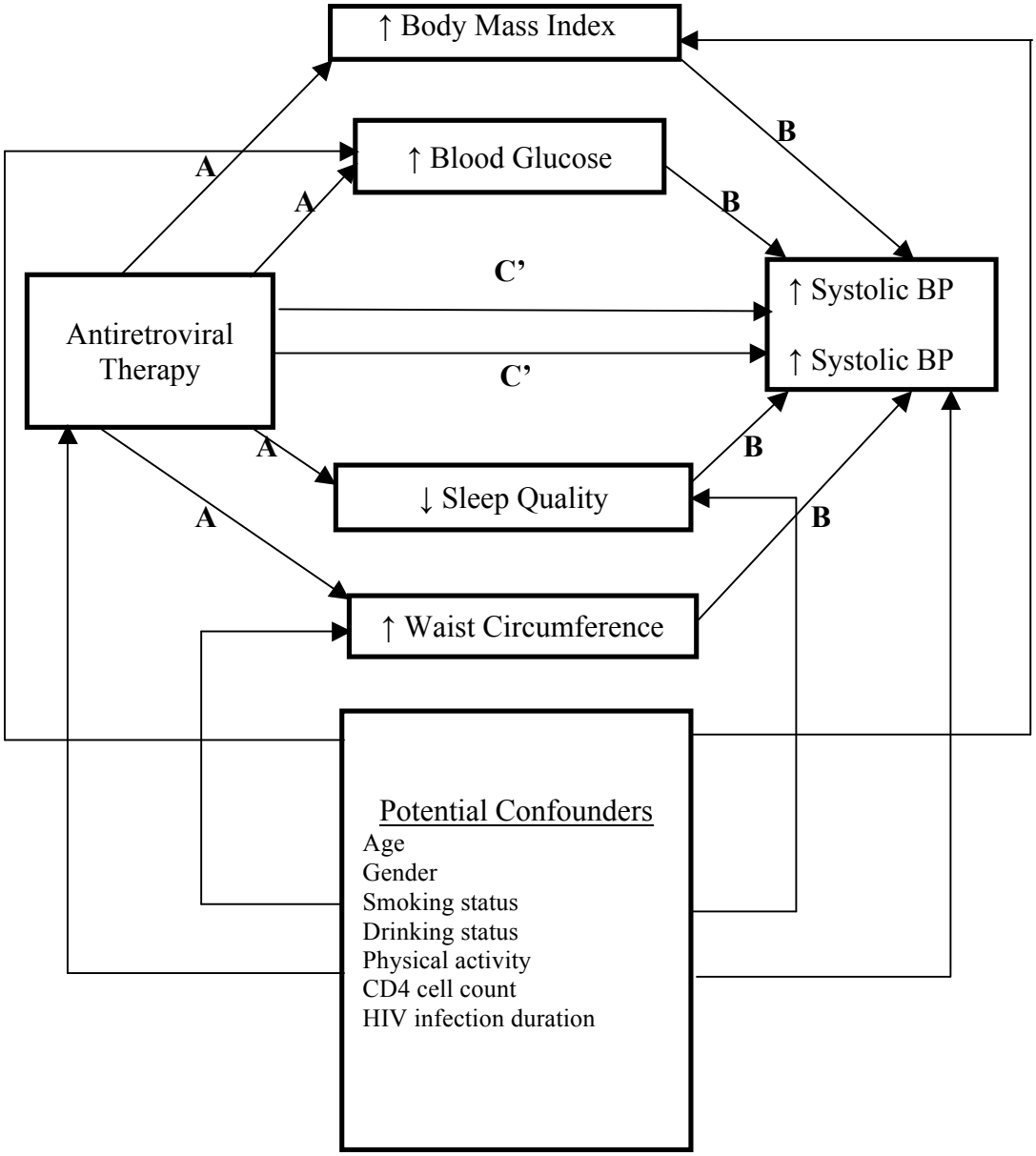
III. Measures of relative weight (such as body mass index) and body fat distribution (such as waist circumference) mediate the total effects of antiretroviral therapy on systolic and diastolic blood pressure.

IV. Blood glucose level mediates the total effects of antiretroviral therapy on systolic and diastolic blood pressure.

V. Sleep quality mediates the total effects of antiretroviral therapy on systolic and diastolic blood pressure.

VI. The average treatment effects of antiretroviral therapy on systolic and diastolic blood pressure are statistically different from the null effect (not shown in the diagram).

Figure 3.1: A conceptual framework of the study hypotheses



Paths AB shows the indirect effects of antiretroviral therapy on systolic and diastolic blood pressure through body mass index, waist circumference, blood glucose, and sleep quality, controlled for potential confounders.
Path C' shows a direct effect of antiretroviral therapy on systolic and diastolic blood pressure.
Path C (not shown) represents the total effect of antiretroviral therapy on systolic and diastolic blood pressure.

PART B

GLOBAL EVIDENCE SYNTHESIS OF THE ASSOCIATIONS BETWEEN ANTIRETROVIRAL THERAPY AND CARDIOVASCULAR DISEASE RISK FACTORS

I have published parts of this section in the following journals.

I. **Chidozie U Nduka**, Saverio Stranges, Ahmed M Sarki, Olalekan A Uthman. Evidence of increased blood pressure and hypertension risk among people living with HIV on antiretroviral therapy: a systematic review with meta-analysis. *Journal of Human Hypertension* 2016; 30: 355 – 362.

II. **Chidozie U Nduka**, Olalekan A Uthman, Ahmed M Sarki, Saverio Stranges. Impact of antiretroviral therapy on serum lipoprotein levels and dyslipidaemias: A systematic review and meta-analysis. *International Journal of Cardiology* 2015; 199: 307 – 318.

III. **Chidozie U Nduka**, Saverio Stranges, Peter K Kimani, Olalekan A Uthman. Increased relative weight and body fat distribution in persons living with HIV on antiretroviral therapy: a systematic review and meta-analysis. *AIDS Reviews* 2016; 18(4).

IV. **Chidozie U Nduka**, Saverio Stranges, Ahmed M Sarki, Peter K Kimani, Olalekan A Uthman. Is there sufficient evidence for routine diabetes and metabolic syndrome screening in HIV-infected patients on antiretroviral therapy? A meta-analysis. *Diabetes/Metabolism Research and Reviews* (revision under review).

CHAPTER FOUR

BACKGROUND

In this chapter, I set the stage for a series of systematic reviews and meta-analyses conducted to examine the evidence for the associations of antiretroviral therapy with increased blood pressure and other cardiovascular disease risk factors in people living with HIV worldwide. I go on to justify the rationale and outline the objectives of the systematic reviews and meta-analyses.

4.1 BACKGROUND

The adverse cardio-metabolic effects of antiretroviral drugs have been well-documented (Barbaro, 2003; Carr & Cooper, 2000; Currier, 2015; Dagogo-Jack, 2008; Dau & Holodniy, 2008; Grinspoon, 2009). Although there have been various primary studies evaluating the impact of antiretroviral therapy on cardio-metabolic changes in HIV-infected patients, the reported effects have been rather inconsistent, with certain studies showing statistically significant associations of antiretroviral exposure with worsening cardiovascular effects (Grandominico & Fichtenbaum, 2008; Thiebaut *et al*., 2005; Shapiro *et al*., 2012) and others showing no significant associations (Bergersen *et al*., 2003; Medina-Torne *et al*., 2012; Wilson *et al*., 2009). In addition, there is no systematic evidence providing comprehensive estimates of the associations between antiretroviral therapy and cardiovascular disease risk factors in people living with HIV worldwide. The systematic reviews and meta-analyses described in this chapter fills this gap in the current body of evidence. In addition, I sought to examine factors that may potentially influence the associations between antiretroviral therapy and cardiovascular disease risk factors.

4.2. RATIONALE FOR METHODOLOGY

A systematic review with meta-analysis entails pooling data from several quantitative primary studies — often with disparate findings — to provide a comprehensive and more consistent evidence that is of a higher level, compared to the individual primary studies (Hemingway & Brereton, 2009). The reasons for combining the results from multiple individual studies using systematic reviews with meta-analyses have been well established (Higgins & Green, 2011; Mulrow, 1994). For instance, a systematic review with meta-analysis is appropriate to obtain pooled estimates of the impact of antiretroviral therapy on cardiovascular risk factors in people living with HIV. Given that HIV-infected adult patients with reported antiretroviral treatment status represent a very specific population, the sample sizes of individual primary studies addressing this question are likely to be too small to detect a statistically significant effect. By performing a meta-analysis, the statistical power — which is the chance of detecting an effect as statistically significant if it truly exists — is increased (Higgins & Green, 2011). Performing meta-analysis would also improve precision, where the estimate of the pooled association is less variable (Higgins & Green, 2011). Furthermore, one cannot make any conclusive inferences if the effect estimates from individual primary studies suggest different interpretations of the associations between antiretroviral therapy and cardiovascular disease risk factors. Such inconsistencies are appropriately resolved by performing a meta-analysis of the individual effect (Higgins & Green, 2011; Mulrow, 1994).

4.3 AIMS AND OBJECTIVES

4.3.1 Aims

I. To estimate the overall impact of antiretroviral therapy on cardiovascular disease risk factors in people living with HIV worldwide.

II. To examine factors that may potentially influence the epidemiological associations between antiretroviral therapy and cardiovascular disease risk factors in people living with HIV worldwide.

4.3.2 Objectives

I. To develop a search strategy for identifying potentially relevant studies examining the associations between antiretroviral therapy and cardiovascular disease risk factors in people living with HIV.

II. To select eligible studies based on pre-defined inclusion and exclusion criteria.

III. To appraise the methodological quality of each included study.

IV. To pool the associations between antiretroviral therapy and cardiovascular disease risk factors by conducting meta-analyses.

V. To perform tests for small-study effects in order to account for evidence (or lack thereof) of publication bias.

VI. To perform sensitivity analyses in order to ascertain whether the pooled associations between antiretroviral therapy and cardiovascular disease risk factors were influenced by individual studies.

VII. pool the associations between antiretroviral therapy and cardiovascular disease risk factors by different study-level characteristics and perform meta-regression analyses to identify potential effect-modifiers of the pooled associations.

VIII. To interpret and summarise the results with due consideration of the strengths and limitations.

CHAPTER FIVE

METHODS

In this chapter, I present the methods of the systematic reviews and meta-analyses, including the search strategies, quality assessment criteria for selected studies, data synthesis, subgroup analysis, meta-regression analysis, sensitivity analysis and analysis of publication bias. I also describe the rationales for these methods.

5.1 PROTOCOL FOR SYSTEMATIC REVIEW AND META-ANALYSIS

The study background, rationale, and methods were specified in advance and documented in a study protocol registered in the PROSPERO database (CRD42014008855). The systematic review and meta-analysis methods are reported in accordance with the preferred reporting items for systematic review and meta-analysis (PRISMA) guideline.

5.2 DATA SOURCES AND SEARCH STRATEGY

Studies eligible for the systematic review and meta-analysis were sought from Embase, MEDLINE (OVID) and the Scientific Electronic Library Online (SciELO). The search was limited to articles published between 1 January 1997 and 22 March 2016. The electronic searches were conducted using the following medical subject heading (MeSH) terms and keywords, while controlling for different spellings and using appropriate truncations and 'wildcards': exp *HIV/, HIV infect$.mp./, HIV-1.mp./, exp *human immunodeficiency virus 1/, exp *human immunodeficiency virus/, exp *human immunodeficiency virus infection/, exp *highly active antiretroviral therapy/, antiretroviral therapy.mp./, exp *antiretrovirus agent/, antiretroviral treatment$.mp./, protease inhibitor$.mp./, exp *proteinase inhibitor/, HAART-naive.mp./, non-nucleoside

reverse transcriptase inhibitor$.mp./, exp *nonnucleoside reverse transcriptase inhibitor/, exp *blood pressure/, exp *systolic blood pressure/, exp *diastolic blood pressure/, exp *hypertension/, exp *lipid/, exp *lipoprotein/, exp *dyslipidemia/, dyslipid?emia$.mp./, exp *hyperlipidemia/, total cholesterol.mp./, exp *cholesterol blood level/, LDL cholesterol.mp./, exp *low density lipoprotein cholesterol/, HDL cholesterol.mp./, exp *high density lipoprotein cholesterol/, triglyceride$.mp./, exp *triacylglycerol/, exp *hypercholesterolemia/, hypercholesterol?emia$.mp./, hypertriglycerid?emia.mp./, exp *hypertriglyceridemia/, exp *blood glucose/, exp *glucose blood level/, exp *diabetes mellitus/, exp *type II diabetes mellitus/, waist circumference$.mp./, exp *obesity/, exp *waist circumference/, exp *body fat/, exp *anthropometry/, exp *adipose tissue/, central obesity.mp/, exp *metabolic syndrome X/. All MeSH terms were exploded — as denoted by adding 'exp' — to retrieve more specific terms.

The search terms were combined using Boolean operators: MeSH terms and keywords of HIV infection, antiretroviral therapy, and cardiovascular disease risk factors were each combined using the 'OR' Boolean operator, and all three groups were combined subsequently using the 'AND' Boolean operator. The database searches were limited to studies on adult humans published after December 1996 in the English language. Boxes 5.1 and 5.2 present examples of the EMBASE and Medline (OVID) search histories. Additional studies were also sought by scanning through the bibliographies of relevant articles identified from the database search.

Box 5.1: Embase search history

Database: Embase Classic+Embase <1947 to 2016 Week 08>
1 HIV.mp. or exp *Human immunodeficiency virus/ (319197)
2 HIV-1.mp. or exp *Human immunodeficiency virus 1/ (88618)
3 exp *Human immunodeficiency virus infection/ or HIV-infect$.mp. (266345)
4 exp *highly active antiretroviral therapy/ (9132)
5 antiretroviral therapy.mp. (55235)
6 exp *antiretrovirus agent/ or antiretroviral treatment$.mp. (74113)
7 protease inhibitor$.mp. or exp *proteinase inhibitor/ (130916)
8 non-nucleoside reverse transcriptase inhibitor$.mp. or exp *nonnucleoside reverse transcriptase inhibitor/ (10305)
9 exp *blood pressure/ (119692)
10 exp *systolic blood pressure/ (4764)
11 exp *diastolic blood pressure/ (2755)
12 exp *hypertension/ (271699)
13 exp *lipid/ or exp *lipoprotein/ or exp *dyslipidemia/ or dyslipid?emia$.mp. or exp *hyperlipidemia/ (698329)
14 total cholesterol.mp. or exp *cholesterol blood level/ (64912)
15 LDL cholesterol.mp. or exp *low density lipoprotein cholesterol/ (33560)
16 HDL cholesterol.mp. or exp *high density lipoprotein cholesterol/ (38872)
17 triglyceride$.mp. or exp *triacylglycerol/ (133822)
18 exp *hypercholesterolemia/ or hypercholesterol?emia$.mp. (67835)
19 hypertriglycerid?emia.mp. or exp *hypertriglyceridemia/ (24171)
20 blood glucose.mp. or exp *glucose blood level/ (111595)
21 diabetes mellitus.mp. or exp *diabetes mellitus/ (737196)
22 body mass index.mp. (168936)
23 waist circumference$.mp. or exp *obesity/ or exp *waist circumference/ or exp *body fat/ or exp *anthropometry/ or exp *adipose tissue/ (245654)
24 exp *metabolic syndrome X/ or central obesity.mp. (32453)
25 1 or 2 or 3 (380677)
26 4 or 5 or 6 or 7 or 8 (223953)
27 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 (2038115)
28 25 and 26 and 27 (6332)
29 limit 28 to (human and english language and yr="1996 - 2016" and (adult <18 to 64 years> or aged <65+ years>)) (2826)

Box 5.2: MEDLINE (OVID) search history

Database: Ovid MEDLINE(R) <1946 to March Week 3 2016>
1 HIV infection$.mp. or exp *HIV Infections/ (235290)
2 exp *Antiretroviral Therapy, Highly Active/ or antiretroviral therapy.mp. (33964)
3 protease inhibitor$.mp. or exp *Protease Inhibitors/ (122448)
4 exp *HIV/ or exp *Anti-HIV Agents/ or exp *Reverse Transcriptase Inhibitors/ or exp *HIV Protease Inhibitors/ or non-nucleoside reverse transcriptase inhibitor$.mp. or exp *HIV-1/ (100639)
5 exp *Blood Pressure/ (76277)
6 exp *Hypertension/ or systolic blood pressure.mp. (190644)
7 diastolic blood pressure.mp. (24671)
8 dyslipid?emia$.mp. or exp *Dyslipidemias/ (60848)
9 hypercholesterol?emia$.mp. or exp *Hypercholesterolemia/ (36471)
10 hypertriglycerid?emia.mp. or exp *Hypertriglyceridemia/ (11851)
11 exp *Triglycerides/ or exp *Lipids/ or exp *Lipoproteins/ or exp *Cholesterol/ or total cholesterol.mp. or exp *Cholesterol, HDL/ (622164)
12 exp *Cholesterol, LDL/ (5755)
13 exp *Blood Glucose/ (41588)
14 exp *Diabetes Mellitus/ (271317)
15 exp *Body Mass Index/ (14024)
16 exp *Body Composition/ or waist circumference$.mp. or exp *Obesity/ or exp *Waist Circumference/ or exp *Adipose Tissue/ or exp *Anthropometry/ (233766)
17 central obesity.mp. or exp *Obesity, Abdominal/ (3803)
18 body fat measure$.mp. or exp *Adiposity/ (3886)
19 exp *Diabetes Mellitus, Type 2/ or exp *Metabolic Syndrome X/ or metabolic syndrome.mp. (106782)
20 2 or 3 or 4 (227221)
21 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 (1371227)
22 1 and 20 and 21 (2782)
23 limit 22 to (english language and humans and yr="1996 - 2016" and "all adult (19 plus years)") (1590)

5.3 CRITERIA FOR SELECTING ELIGIBLE STUDIES

5.3.1 Inclusion criteria

Studies with the following characteristics were included in the systematic review and meta-analyses:

I. *Participants*: HIV-infected adults at least 18 years of age.

II. *Exposure criterion*: highly active antiretroviral therapy (a study was considered eligible if it had both antiretroviral-exposed and antiretroviral-naïve patients).

III. *Outcomes*: systolic blood pressure, diastolic blood pressure, hypertension, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, hypercholesterolaemia, hypertriglyceridaemia, combined dyslipidaemia, fasting plasma glucose levels, diabetes mellitus (Types 1 and 2), body mass index, waist circumference, combined overweight/obesity, central obesity and metabolic syndrome.

IV. *Study type*: cross-sectional studies, case-control studies, cohort studies, randomised controlled trials, abstracts, published data, and studies published after 1996: the year marking the advent of HAART (Hooshyar *et al*., 2007)

5.3.2 Exclusion criteria

Studies excluded from the systematic review and meta-analysis had the following characteristics:

I. *Participants*: HIV-negative individuals, HIV-positive adolescents and children.

II. *Exposure criterion*: a study was excluded from the search if it did not include antiretroviral-exposed and naïve participants.

III. *Outcomes*: coronary heart diseases and strokes were outside the scope the thesis.

IV. *Study type*: expert reviews, policy reports, unpublished data and studies published prior to 1996.

5.4 QUALITY ASSESSMENT IN EACH INCLUDED STUDY

Appraising the methodological quality of each included study entailed assessing the risks of bias in each study using a checklist adapted after the Cochrane risk of bias tool for non-randomised studies (Sterne, Higgins & Reves, 2014). The risk of bias assessment tool is shown in Box 5.3. Five potential sources of bias were explored: selection of participants (selection bias), assessment of exposure (information bias), assessment of the outcomes (information bias), adjustment for confounding, and follow-up of participants (attrition bias—applicable only to randomised controlled trials and cohort studies).

Box 5.3: Risk of bias assessment tool

Domain	Assessment criteria
Sampling of participants (Selection bias)	*Low risk:* Participants were all included or sampled randomly Similar recruitment strategy for ART-exposed and naïve participants The sample size was justified *High risk:* Participants were not selected randomly ART-exposed and naïve participants recruited differently The sample size was not justified *Unclear risk*: Sampling strategy was not described The sample size was not calculated
Assessment of exposure (Information bias)	*Low risk*: Exposure criterion well defined, in which case, ART-naïveté only refers to patients who have never been exposed to ART *High risk*: Exposure criterion not well defined, e.g. studies may define ART exposure based on treatment duration of at least one month; this misclassifies certain ART- exposed patients as ART-naïve *Unclear risk*: Exposure criterion not clearly stated
Assessment of outcomes (Information bias)	*Low risk*: Cardiovascular risk factors assessed similarly between ART-exposed and ART-naïve participants Cardiovascular risk factors measured using a validated tool *High risk*: Cardiovascular risk factors not assessed similarly between ART-exposed and ART-naïve participants Cardiovascular risk factors not measured using a validated tool *Unclear risk*: Methods of measuring cardiovascular risk factors not described
Adjustment for confounding	*Low risk*: Adjusted for at least one major confounder *High risk*: No attempt at adjusting for confounders
Follow-up of participants – for cohort studies and RCTs (Attrition bias)	*Low risk:* Less than 20% loss to follow-up *High risk*: Loss to follow-up ≥ 20%

Adapted from Sterne, Higgins & Reves (2014). ART, antiretroviral therapy; RCTs, randomised controlled trials

5.5 DATA EXTRACTION

Data on study characteristics and outcome measures were extracted from each study using a data extraction form based on guidance by the University of York Centre for Reviews and Dissemination (2008) (Appendix 1). The extracted data included: article citation, country of origin, country income group, geographical region, study design, sample size, mean age, proportion of females, proportions of current smokers and drinkers, mean duration of antiretroviral therapy, antiretroviral regimen, numbers of patients exposed or naïve to antiretroviral therapy, and the mean CD4 (cluster of differentiation 4) cell counts. Of note, CD4 cells, also called T4 cells or CD4+ T cells, are white blood cells that fight infection, and a CD4 count is the number of CD4 cells in a sample of blood. In other words, the CD4 count is a measure of the immune status of an individual, where higher CD4 counts correspond to higher levels of immunity against infections and vice versa. However, the human immunodeficiency virus specifically targets these CD4 cells and destroys them, consequently lowering the CD4 cell count as well as the body's innate ability to fight infections (AIDS Info, 2009).

Data on outcome measures for antiretroviral-exposed and antiretroviral-naïve patients were also extracted: mean systolic and diastolic blood pressure levels, hypertension prevalence, mean serum lipoprotein levels, prevalence estimates of hypercholesterolemia hypertriglyceridemia and combined dyslipidaemia, mean fasting blood glucose concentration, mean body mass index, mean waist circumference, prevalence estimates of combined overweight/obesity and central obesity, and prevalence estimates of metabolic syndrome.

Country income groups were defined according to World Bank development indicators (The World Bank Group, 2016). Hypertension was defined as blood pressure of at least 140/90 mmHg or the use of antihypertensive medication (World Health Organisation, 1999). Hypercholesterolemia was defined as serum total cholesterol levels no less than 240 mg/dL and hypertriglyceridemia as serum triglyceride levels no less than 150 mg/dL (Adult Treatment Panel III, 2001). Diabetes mellitus was defined as fasting blood glucose concentration no less than 7 mmol/L (126 mg/dL) or a two-hour post-prandial blood glucose level no less than 11.1 mmol/L (200 mg/dL) (World Health Organization & International Diabetes Federation, 2006). Combined overweight/obesity was defined as body mass index no less than 25 kg/m^2, whereas abdominal obesity was assessed as waist circumference no less than 80 cm for women and no less than 94 cm for men (National Institute for Health and Clinical Excellence, 2011). Metabolic syndrome was defined as any combination of three or more cardio-metabolic abnormalities (Kaur, 2014).

5.6 SUMMARY MEASURES

For continuous outcome variables, effect was quantified using the mean difference and meta-analysis provides the pooled mean difference. For dichotomous categorical variables, effect was quantified using the odds ratio and meta-analysis provides the pooled odds ratio. All data were analysed using Stata version 14 for Windows (Stata Corp, College Station, Texas).

5.7 DATA SYNTHESIS

Due to the anticipated differences in characteristics between individual studies, meta-analysis was performed using the random-effects model proposed by DerSimonian & Laird (1986). For instance, in the proposed meta-analysis, the effect size might be of greater magnitude among HIV-infected patients who are older, or have lower CD4 cell

counts, or on protease inhibitor-based antiretroviral treatment etc. Using a random effects model, the individual studies in the meta-analysis are assumed to represent a random distribution of the individual effect sizes, so that the true effect size varies between these studies, and the pooled effect estimate represents the mean effect size in this distribution. In contrast, a fixed-effect meta-analysis assumes that there are no characteristic differences between studies, so that any observed heterogeneity in the results are entirely due to chance (Higgins & Green, 2011). In a fixed-effect model, it is assumed that there is one true effect size that is common to all the included studies in a fixed effect meta-analysis, in which case the pooled effect is an estimate of this common effect size (Borenstein, Hedges & Rothstein, 2007). The implication of these differences with regard to estimating the pooled effect is that studies with relatively larger sample sizes may be assigned significantly more weight and small studies could be assigned weights that are potentially negligible in fixed-effect meta-analyses; whereas, the weight assigned to studies pooled using random effects meta-analyses tend to be more balanced, so that larger studies are less likely to drive the pooled effect estimates and smaller studies are less likely to be ignored (Borenstein, Hedges & Rothstein, 2007).

5.8 EXPLORING HETEROGENEITY ACROSS INCLUDED STUDIES

Heterogeneity across studies included in the meta-analyses was measured using the chi-squared (chi^2) test for heterogeneity, and at a statistical significance level of 5% (Higgins *et al*., 2003). It is worth noting that the chi^2 test for heterogeneity is often influenced by the number of studies included in a meta-analysis. For instance, a meta-analysis of a small number of studies would not be adequately powered to detect statistically significant heterogeneity in the effect estimates across the included studies, whereas when there are many studies included in a meta-analysis, the chi^2 test for heterogeneity is adequately

powered to detect even a small amount of statistical heterogeneity in the effect estimates that may not be clinically significant (Higgins & Green, 2011). However, a chi^2 statistic that is statistically non-significant may not necessarily indicate the absence of heterogeneity, given that methodological differences between studies in any meta-analysis are bound to occur (Higgins *et al*., 2003). Nevertheless, the chi^2 test for heterogeneity may be complemented with the *I*-squared (I^2) statistic, which measures the proportion of between-study variability in the effect estimates that accounts for heterogeneity across the pooled studies (Higgins & Green, 2011). The I^2 statistic, which is often reported in the forest plot when computing a meta-analysis, is calculated using the formula: $I^2 = (^{Q - df}/_{Q}) \times 100\%$, where Q is the chi^2 statistic and df stands for the degree of freedom (Higgins & Thompson, 2002; Higgins *et al*., 2003). In the present meta-analysis, an I^2 statistic explaining no less than 75% of between-study variability in the effect estimates implied considerable heterogeneity (Higgins & Green, 2011; Higgins & Thompson, 2002).

5.9 SUBGROUP ANALYSIS

To investigate how study-level characteristics influenced the impact of antiretroviral therapy on cardio-metabolic effects in the meta-analyses, the studies included in each meta-analysis were divided according to categories or subgroups of each study-level characteristic as follows:

I. *Region* – Each of the five subgroups within this study-level characteristic corresponded to a particular region, so that estimates from studies conducted with HIV-infected populations in sub-Saharan African countries were pooled as one subgroup, and the other four subgroups comprised studies of HIV-infected populations in Europe, South-East Asia, The Americas and Western Pacific respectively.

II. *Country income group* – The two subgroups within this study-level characteristic included studies conducted in high-income countries, and studies conducted in low- and middle-income countries (The World Bank Group, 2016).

III. *Age group* – Subgroups included studies in which the mean age of the participants was 40 years or more, and studies in which the mean age was less than 40 years. The cut-off age of 40 years was based on previous studies that found age above 40 years to be an important predictor of cardiovascular disorders among people living with HIV (Awotedu *et al*., 2015; Kagaruki *et al*., 2014; Kaplan *et al*., 2007; Petoumenos *et al*., 2014).

IV. *Gender* – Subgroups included studies with fewer women (< 50%) than men, and studies with more women than men.

V. *Smoking status* – Subgroups included studies with fewer smokers (< 50%) than non-smokers, and studies with more smokers than non-smokers.

VI. *CD4 cell count* – Subgroups included studies in which the mean CD4 count of all HIV-infected patients was no greater than 350 cells/mm^3, and studies in which the mean CD4 count was greater than 350 cells/mm^3. The cut-off CD4 cell count of 350 cells/mm^3 represents the threshold below which antiretroviral therapy must be initiated to significantly reduce HIV-related morbidity and mortality (World Health Organization, 2013). Although more recent evidence suggests that initiating antiretroviral therapy at a higher cut-off value of 500 cells/mm^3 may confer additional benefits, including reduced incidence of certain opportunistic infections, notably pulmonary tuberculosis, and decreased risk of sexual transmission of HIV infection (World Health Organization, 2016), this threshold is yet to be adopted in many low- and middle-income countries.

VII. *Duration of HIV infection* – Subgroups included studies in which the mean duration of HIV infection was less than 60 months, and studies in which mean HIV duration was no less than 60 months. In a secondary analysis of data from eight cohorts comprising over

18,000 HIV-infected patients, Lyons *et al*. (2015) found a significant increase in the risk of cardiovascular disease for every five years of exposure, and independent of age, hence the choice of this threshold in the present study.

VIII. *Antiretroviral regimens* – Subgroups included studies in which HIV-infected patients received protease inhibitor-based treatment regimens, and studies in which all HIV-infected participants received other antiretroviral regimens.

IX. *Duration of antiretroviral therapy* – Subgroups included studies in which the mean duration of antiretroviral therapy was less than 18 months, and studies in which mean antiretroviral treatment duration was no less than 18 months. Lai *et al*. (2009) found that the risk of cardiovascular disease was increased more than seven folds among HIV-infected patients who had been on antiretroviral therapy for longer than 18 months, compared to HIV-infected patients who had received antiretroviral therapy for less than 18 months, hence the choice of this threshold in the present study.

X. *Study design* – Each subgroup comprised studies of a certain design, e.g. cross-sectional studies, cohort studies etc.

XI. *Sample size* – An arbitrary cut-off of 200 participants was used to divide the studies into subsets, so that the subgroups comprised studies with 200 participants or more, and studies with less than 200 participants.

XII. *Year of publication* – Subgroups included studies published prior to 2010, and studies published from 2010 onwards. The selection of 2010 as the basis for grouping the studies into subsets was arbitrary.

5.10 STUDY-LEVEL FACTORS ASSOCIATED WITH THE POOLED ESTIMATES (META-REGRESSION ANALYSIS)

Random-effects meta-regression analyses were performed on all study-level characteristics to examine for potential effect-modifiers of the pooled associations between antiretroviral therapy and cardiovascular disease risk factors. Random-effects meta-regression, as opposed to the fixed-effect model, was appropriate to account for any unexplained heterogeneity in the effect estimates (Higgins & Green, 2011; Higgins & Thompson, 2002). In investigating potential sources of heterogeneity across the included studies, meta-regression analyses also identified study-level variables that modified the pooled association.

The degree to which such study-level variables fit within the meta-regression models corresponds to the amount of variability in the effect estimate between the included studies, and is denoted R-squared (R^2). Meta-regression models are quite similar to the ordinary least squares (OLS) regression models in the sense that the outcome variables (the pooled effect estimates) are regressed on the predictor variables (subgroups of the study-level characteristics or potential effect-modifiers) (Higgins & Green, 2011).

The results revealed whether the differences between subgroup estimates of the pooled associations — as determined in the subgroup analyses — were statistically significant. In other words, meta-regression analyses were an extension of the subgroup analyses, so that the results of these analyses were presented simultaneously (Thompson & Higgins, 2002). Where the effect estimate in the meta-regression model was an odds ratio, the log-transformed value of the effect estimate was regressed on each study-level characteristic. The statistical significance of the subgroup differences in the pooled

associations were put at 5% significance level, so that the pooled association between antiretroviral therapy and each cardio-metabolic outcome was modified by a study-level characteristic when the *P* value for interaction between subgroup estimates was less than 0.05. In accordance with standard practice, meta-regression analyses were only performed when there were ten or more studies, in order to effectively examine potential effect-modifiers (Higgins & Green, 2011; Higgins & Thompson, 2002; Thompson & Green, 2002).

5.11 SENSITIVITY ANALYSIS

Leave-one-out sensitivity analyses were performed to investigate the validity and robustness of the meta-analyses: sensitivity analyses ensure that the findings of a meta-analysis are not dependent on any particular study. Essentially, the included studies were omitted one at a time, while iteratively computing the meta-analyses. For each study outcome, meta-analysis was considered valid and robust if the confidence intervals of all the pooled effect estimates did not change considerably as to alter the interpretation of the result following serial omission of the included studies (Higgins & Green, 2011).

It is worth noting that the results for each sensitivity analysis undertaken were presented in a summary table. In fact, the Cochrane Collaboration stipulates that the results of sensitivity analyses be summarised in tabular formats, and considers it redundant to compute individual forest plots for each sensitivity analysis (Higgins & Green, 2011).

5.12 ANALYSIS OF PUBLICATION BIAS

Rothstein, Sutton & Borenstein (2005) emphasize that publication bias must be addressed as its presence arguably poses the single greatest threat to the validity of the results in a meta-analysis. Publication bias was investigated using the Egger's regression test for

small-study effects, complemented by visual interpretations of funnel plots. A funnel plot is a scatterplot of the sample size of each included study in a meta-analysis against the effect estimate of that study (Egger *et al*., 1997; Sterne *et al*., 2011). Generally, larger studies tend to have more precise estimates of the effect size, compared to smaller studies, so that the effect estimates from smaller studies are scattered widely at the bottom of the plot, whereas, estimates from larger studies tend to have a narrower spread (Sterne & Harbord, 2004). Therefore, in the absence of publication bias, the plot resembles a symmetrical inverted funnel. Conversely, the presence of publication bias, for instance, when smaller studies showing no statistically significant associations between antiretroviral therapy and cardiovascular risk parameters are not included in the meta-analysis because they remain unpublished, results in an asymmetrical appearance of the inverted funnel. The more pronounced the funnel plot asymmetry, the more likely it is that publication bias is substantial (Sterne & Harbord, 2004). However, funnel plot inspection is innately subjective because it is prone to observer error. On the other hand, the Egger's test for small study effects (or Egger's regression test for funnel plot asymmetry) examines whether the association between the effect estimates and the sample size is greater than what is expected to occur by chance, in which case, the *P* value for Egger's regression test at 5% significance level would be less than 0.05 in the presence of publication bias, and vice versa in the absence of publication bias (Harbord, Harris & Sterne, 2009).

Where publication bias was present, its potential impact on each pooled estimate was ascertained by imputing 'missing' studies and re-computing the pooled effect estimate using Stata's *metatrim* command. This method of adjusting the meta-analysis for publication bias is termed the 'trim and fill' analysis of publication bias, proposed by

Duval and Tweedie (2000). Adding the imputed 'missing' studies to the original dataset creates an 'unbiased' dataset (or a trimmed and filled dataset). The pooled effect estimate of the 'unbiased' dataset should not be significantly different from the pooled estimate of the original dataset in the sense that the overall interpretation of the association between antiretroviral therapy and cardiovascular disease risk factors is not altered. A funnel plot of the unbiased dataset should also appear more symmetrical than the original data. Conversely, if the differences in the pooled estimates between the original and unbiased dataset are significantly different, then it becomes evident that the pooled association was influenced by publication bias (Steichen, Egger & Sterne, 1998).

CHAPTER SIX

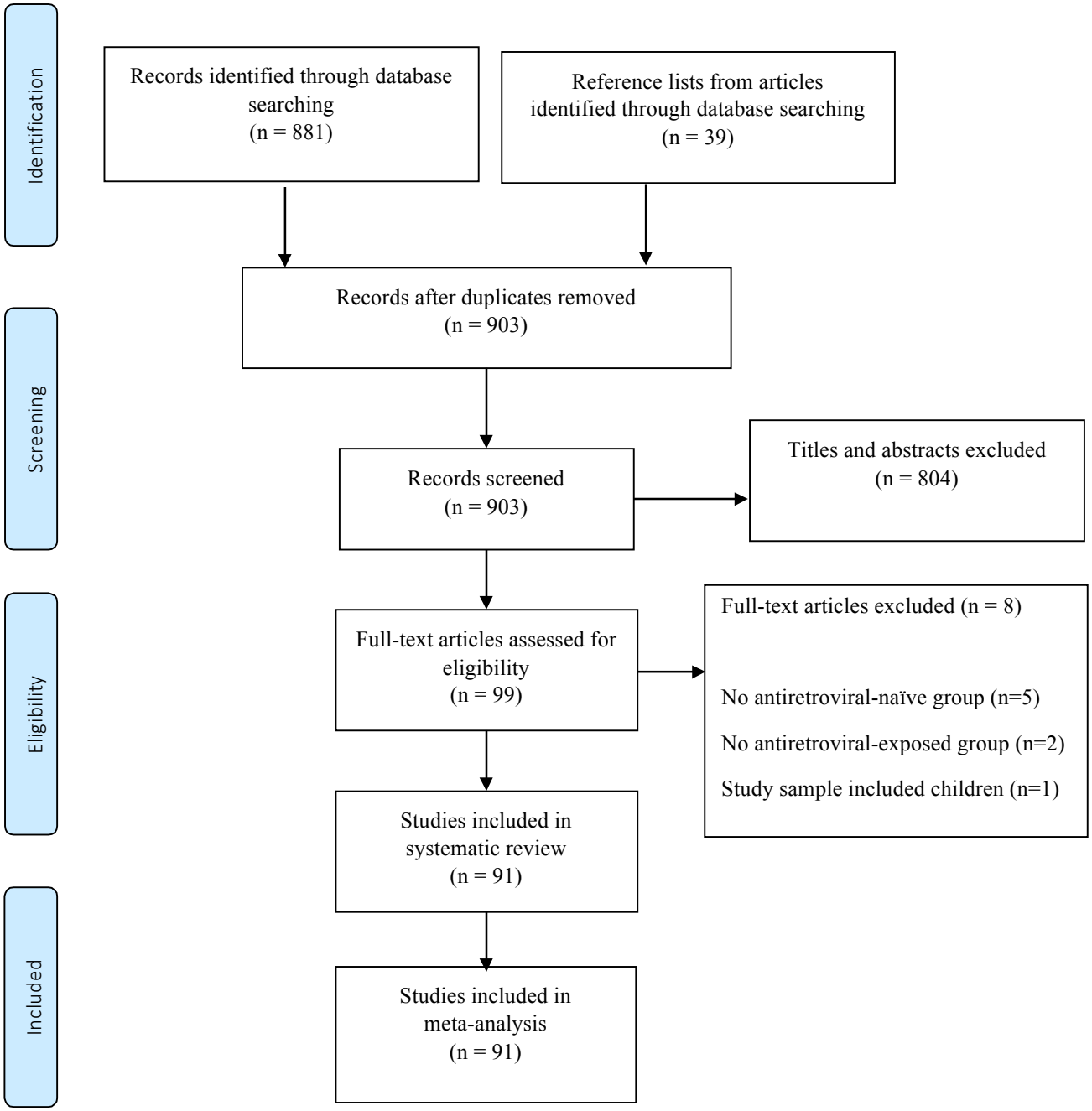
RESULTS – DESCRIPTION OF THE INCLUDED STUDIES

In this chapter, I describe the results of the search strategies, characteristics of the included studies, risk of bias assessment within each included study.

6.1 STUDY SELECTION

The details of the study selection process are illustrated in the flow diagram shown in Figure 6.1. From 920 records yielded by the database search (881 records) and cross-references of articles identified from database searching (39 records), 804 articles were excluded by their titles and abstracts and 17 duplicate records were withdrawn. Of the 99 remaining articles assessed to determine eligibility for inclusion, eight additional articles were withdrawn after reviewing the full texts (Ahoua *et al*., 2011; Feleke, Fekade & Mezegebu, 2012; Gedefaw *et al*., 2013; Kinabo *et al*., 2013; Mercier *et al*., 2009; Nzou *et al*., 2010; PrayGod *et al*., 2011; Scarcella *et al*., 2011), leaving 91 studies considered to be eligible for inclusion in the systematic review and meta-analyses.

Figure 6.1: Flow diagram showing study selection



6.2 CHARACTERISTICS OF THE INCLUDED STUDIES

Table 6.1 summarizes the baseline characteristics of all 91 studies, which comprised a total of 83,669 HIV-positive patients. Overall, female participants accounted for 42% of the meta-analysis study population; mean age of the study population was 39.1 ± 9.3 years; proportions of current smokers and current drinkers were 31% and 17% respectively; mean CD4 cell count was 341 ± 178 cells/mm^3; proportion with one or more AIDS-defining illnesses was 34%; mean duration of HIV infection was 66.4 ± 43.9 months; mean duration of antiretroviral therapy was 32.4 ± 16.1 months; proportion of antiretroviral-exposed patients receiving protease inhibitor-based antiretroviral regimen was 28%; and there were more than twice as many antiretroviral-exposed patients as there were antiretroviral-naïve patients.

Thirty-three of the 91 included studies were conducted in sub-Saharan African countries, including Benin (Zannou *et al*., 2009), Botswana (Shapiro *et al*., 2012), Cameroon (Dimodi *et al*., 2014; Ekali *et al*., 2013; Mbunkah *et al*., 2014; Ngondi *et al*., 2007), Ethiopia (Abebe *et al*., 2014; Mohammed *et al*., 2015; Tadewos *et al*., 2012; Tesfaye *et al*., 2014), Ghana (Ngala & Fianko, 2013; Owiredu *et al*., 2011), Kenya (Manuthu *et al*., 2008); Nigeria (Adewole *et al*., 2010; Akinboro *et al*., 2013; Ayodele *et al*., 2013; Denue *et al*., 2012; Denue *at al*., 2013; Denue, Ikunaiye & Denue, 2013; Muhammad, Sani & Okeahialam, 2013a; Muhammad, Sani & Okeahialam, 2013b; Mustapha *et al*., 2011; Ogundahunsi *et al*., 2008; Ogunmola *et al*., 2014), South Africa (Abrahams *et al*., 2015; Awotedu *et al*., 2010; Esposito *et al*., 2008; Goedecke *et al*., 2013; Jaff *et al*., 2015), Tanzania (Peck *et al*., 2014), Uganda (Buchacz *et al*., 2008; Scholten *et al*., 2011), and Zambia (Kiage *et al*., 2013). However, studies conducted in European countries (n = 27) were generally larger in size (N = 50,683 participants) compared to studies from the other

geographical regions. Patients from sub-Saharan African countries were younger than patients from other geographical regions, and were mostly females.

The reported outcomes included: systolic blood pressure, diastolic blood pressure, hypertension, LDL cholesterol, HDL cholesterol, total cholesterol, triglycerides, hypercholesterolaemia, hypertriglyceridaemia, combined dyslipidaemia, fasting blood glucose, diabetes mellitus, body mass index, combined overweight/obesity, waist circumference, central obesity and metabolic syndrome.

Table 6.1: Characteristics of the included studies

Authour	Year	Study design	Country	Income group	Region	N	F (%)	Age (years)	HIV duration (months)	CD4 count (cells/mm^3)	Smokers (%)	Drinkers (%)	AIDS (%)	ART duration (months)	PI (%)
Abebe	2014	Cross-sectional	Ethiopia	Low/middle	SSA	232	72.3	35.3±10	20.7±14.7	364±199					0
Abrahams	2015	Cohort	South Africa	Low/middle	SSA	103	100	33.5		372±52.5				16±4	0
Aboud	2010	Cross-sectional	UK	High	Europe	963	26				37.1				
Adewole	2010	Cohort	Nigeria	Low/middle	SSA	130	69				23	31		7±2.8	0
Akinboro	2013	Cohort	Nigeria	Low/middle	SSA	140	68	35±8.8		288±232			31.5	5.6±2.7	0
Arruda Junior (a)	2010	Case-control	Brazil	Low/middle	America	570	39	39.5±10		314±159	2.7	13.9		49.5±27.4	12
Arruda Junior (b)	2010	Case-control	Brazil	Low/middle	America	633	41.7								
Awotedu	2010	Cross-sectional	South Africa	Low/middle	SSA	196	81	36.9±10.4							
Ayodele	2013	Cross-sectional	Nigeria	Low/middle	SSA	265	67.5	38.7±8.7		313±230	1.9	7.2		17.3±11	0
Baekken	2008	Cohort	Norway	High	Europe	542	27.1	42.9±9.9							
Bajaj	2013	Cross-sectional	India	Low/middle	S/E Asia	70	28.6							49.5±27	
Bergersen	2003	Cross-sectional	Norway	High	Europe	283	20	43.1±10.2	77±56	384±206	54.5		30	33±2	
Bergersen	2004	Cross-sectional	Norway	High	Europe	283	20	42.7±10.2						33.6±15.3	
Bergersen	2006	Cross-sectional	Norway	High	Europe	263	19.4	43.1±20.1		313±230	1.9	7.2		2	
Blass	2008	Cross-sectional	Germany	High	Europe	44	18.2	40±7.1		441±265				43.5±17.3	67.9
Blumer	2008	Cohort	Netherlands	High	Europe	39		42.3±7		260±148			18	3	100
Bonfati	2007	Cross-sectional	Italy	High	Europe	1243	28.2	43.2			60.2				
Bonfati	2012	Cohort	Italy	High	Europe	188	24.5	39.5±11.1						18.5±8.8	46.5
Buchacz	2008	Cohort	Uganda	Low/middle	SSA	374	49	39						24	
Calza	2011	Cross-sectional	Italy	High	Europe	755		37							
Carey	2013	Cross-sectional	India	Low/middle	S/E Asia	108	53.8	36.3±7.6	46					32±20.3	
Ceccato	2011	Cohort	Brazil	Low/middle	America	620	33.5	39.2±9.9						28.2±15.5	32.1
Chow	2003	Cohort	USA	High	America	237	11.4	39.5±7.9							
Denue	2012	Cohort	Nigeria	Low/middle	SSA	227	49	40.3±9.3	56±53	246±168	5.3		81.05	24	0
Denue (a)	2013	Cohort	Nigeria	Low/middle	SSA	229	51.1	43.5±9.3		246±166	5.7	4.9	45.9	24	0
Denue (b)	2013	Cohort	Nigeria	Low/middle	SSA	107	68.2	39.4±9.3		229±174			61.67	16.5±6	0
Dimodi	2014	Cross-sectional	Cameroon	Low/middle	SSA	463	74.7				5.05	35.2	8.06		3.2
Domingos	2009	Cross-sectional	Brazil	Low/middle	America	292	40	41±13	46.6		15.4			40.4	60.2
Eira	2012	Cross-sectional	Brazil	Low/middle	America	56		42.8±7.1	101.4±51	328±183	57.14			92.4±40.8	
Ekali	2013	Cross-sectional	Cameroon	Low/middle	SSA	143	72	39.5±9.8		253±167				43.5±21.3	0

Authour	Year	Study design	Country	Income group	Region	N	F (%)	Age (years)	HIV duration (months)	CD4 count (cells/mm^3)	Smokers (%)	Drinkers (%)	AIDS (%)	ART duration (months)	PI (%)
Esposito	2008	Cohort	South Africa	Low/middle	SSA	30	100	30.9±5.6		164±69				3.5±1.3	0
Fontas	2004	Cohort	Multi-centre	High	Europe	7483	24	38±2.5		470±90			19.9	48±8	78.4
Friis-Møller	2003	Cohort	Multi-centre	High	Europe	17852	24	39±2.8		430±88			24.8	26.4±4.1	71.7
Goedecke	2013	Cross-sectional	South Africa	Low/middle	SSA	744	100	33±5.3		371±143				24.5±9	20.9
Gowdaiah	2013	Cohort	India	Low/middle	S/E Asia	100	35	36.2±6.2						2.4	
Grandominico	2008	Cohort	USA	High	America	52	9.6	35.9±10.1		298±163	57.1		14	6	55.5
Hansen	2009	Cross-sectional	Denmark	High	Europe	466	18.6	45.5±10.2	115.2±81.6	519±233			20.3	60±51.5	72.98
Howard	2014	Cross-sectional	UK	High	Europe	100									40.74
Jaff	2015	Cross-sectional	South Africa	Low/middle	SSA	86	100	47±5.1							
Jain	2013	Cross-sectional	India	Low/middle	S/E Asia	85	22.4	34.4±8.3						10.4±5.9	
Jantarapakde	2014	Cross-sectional	Thailand	Low/middle	W/Pacific	580	53.8	37±8.2	60±62.2	406±208	16.3		40.2	37.8±30.2	14.9
Jerico	2005	Cross-sectional	Spain	High	Europe	710	28	42±9.2	110±62.2	489±284	69.5		32.7	70.5±45.9	74.7
Kiage	2013	Cohort	Zambia	Low/middle	SSA	118	55.9	7.9		136±50	4.4	9.6		3	0
Kingsley	2008	Cross-sectional	USA	High	America	615	0						14		
Koethe	2015	Cohort	Multi-centre	High	America	14084	13	40							
Koppel	2000	Cross-sectional	Sweden	High	Europe	340	4.1	40.5±8.5	88.5±54.5	484±236				27±7	
Lekakis	2009	Case-control	Greece	High	Europe	56	4	40±13	42±27.5	452±260	73				73
Levy	2005	Cohort	Canada	High	America	679	9	39±8.9		255±222				45.5±24.4	100
Lin	2011	Cohort	Taiwan	High	W/Pacific	1344		49.5±27							
Magenta	2011	Cohort	Switzerland	High	Europe	74	12	10		258±112	65			20.4±8.3	100
Malapati	2014	Cohort	India	Low/middle	W/Pacific	229		46.3±9.3		246±167				12	0
Maloberti	2013	Cross-sectional	Italy	High	Europe	108		43.6±7.7							
Manuthu	2008	Cross-sectional	Kenya	Low/middle	SSA	290	58.1	37.1							
Mariz	2011	Cross-sectional	Brazil	Low/middle	America	2018	37.86				27	33.9	86.45		
Mbunkah	2014	Cross-sectional	Cameroon	Low/middle	SSA	173	71.1	38.7±11.4		332±168	2.9	8.1			28.9
Medina-Torne	2012	Cross-sectional	USA	High	America	707	8	41±7.4			57.14				
Mital	2013	Cross-sectional	India	Low/middle	S/E Asia	200			35.4±7.9		17.5	11.5			0
Mittal	2013	Cross-sectional	India	Low/middle	S/E Asia	40	32.5	36.1±7.2						39.6±16.5	100
Mohammed	2015	Cross-sectional	Ethiopia	Low/Middle	SSA	393	66.9								
Montes	2005	Cohort	Spain	High	Europe	107	30	47.5±39.3		105±116				53±7	2.5
Muhammad (a)	2013	Cross-sectional	Nigeria	Low/middle	SSA	200	53	32.5±7.6	72.5±35.8	319±206	9			45±19.5	1

Authour	Year	Study design	Country	Income group	Region	N	F (%)	Age (years)	HIV duration (months)	CD4 count (cells/mm^3)	Smokers (%)	Drinkers (%)	AIDS (%)	ART duration (months)	PI (%)
Muhammad (b)	2013	Cross-sectional	Nigeria	Low/middle	SSA	200	53	32.5±7.6	72.5±35.8	319±206	9			45±19.5	1
Mustapha	2011	Cross-sectional	Nigeria	Low/middle	SSA	100		32.9±7.5						45.0±19.5	0
Ngala	2013	Cross-sectional	Ghana	Low/middle	SSA	305	61	38.5±8.7						17.0±6.3	0
Ngondi	2007	Cohort	Cameroon	Low/middle	SSA	138	57.97	36.1±7.1		209±222			13.78	12	0
Ogundahunsi	2008	Cross-sectional	Nigeria	Low/middle	SSA	110		38.7±10.1		355±185				60.3±12	0
Ogunmola	2014	Cross-sectional	Nigeria	Low/middle	SSA	250	62.4	37.6±8.6		374±223					
Owiredu	2011	Cross-sectional	Ghana	Low/middle	SSA	442	74	35.2±17.3							
Palacios	2006	Cohort	Spain	High	Europe	95	18	40±10.1	56.1±57.1	165±125	68		44	12	48
Peck	2014	Cross-sectional	Tanzania	Low/middle	SSA	301	67.8	40.3±7.8		314±159	2.7	13.9		49.5±24.7	12
Pefura Yone	2011	Cross-sectional	USA	High	America	276	60.5	39.7±8.7		308±186				30.2±13.1	0
Samaras	2007	Case-control	Multi-centre	High/middle	Intercontinental	788	16	43.5±9	95.4±54			20.7			50.4
Scholten	2011	Cross-sectional	Uganda	Low/middle	SSA	199	57.4				69.5				
Shahmanesh	2004	Cross-sectional	UK	High	Europe	55	18.2	40±10.4		424±225	41.8			39.8±8.8	37.5
Shapiro	2012	Cross-sectional	Botswana	Low/middle	SSA	62	100	31			41.5				
Silva	2009	Cross-sectional	Brazil	Low/middle	America	319	38.9	39.5	51.6	531.9±422.4	26.7				
Silva	2010	Cross-sectional	Brazil	Low/middle	America	314	44.6	37.7±7.9	51±46.2	531±313	26.7			52.8±42	
Singh	2014	Cohort	India	Low/middle	S/E Asia	100	35	36.4±9.6		151±67				6	0
Smith	2004	Cross-sectional	UK	High	Europe	394	15		84±15	426±87	45	7	30		27
Soares	2015	Cross-sectional	Brazil	Low/middle	America	152	33	42.5±8.5	72±48	444±88				64±48	
Sogaard	2010	Cohort	Denmark	High	Europe	95	15.8	48.7±4.1		571±99	35.8		22.1		
Sreekantamurthy	2014	Cross-sectional	India	Low/middle	S/E Asia	101	0	43.5±6.3	64.9±30.2					67.8±16.5	30.38
Tadewos	2012	Cross-sectional	Ethiopia	Low/middle	SSA	226	65.1	35.5±8.5		408±219	11.5			49.4±16.5	0
Tesfaye	2014	Cross-sectional	Ethiopia	Low/middle	SSA	374	68	32.6							
Thiebaut	2005	Cohort	Multi-centre	High	Europe	16770	24	39.3±8.2		438±260					
van Leth	2004	Cohort	Multi-centre	High	Europe	706	36.8	35±7.4						11.5±2.7	
Wanke	2005	Cohort	USA	High	America	49	14.29	40.6±8		205	35			5.7±1.7	100
Weerakkody	2013	Cross-sectional	Sri Lanka	Low/middle	S/E Asia	268	42.2	39.5±9.6	42.5±36.2	390±243	20.1			33±23	7.3
Wilson	2009	Cross-sectional	UK	High	Europe	458	16.81	39.5±8.9	66±48.8	545±83	41.5	21.8		77.4±33	39
Zannou	2009	Cohort	Benin	Low/middle	SSA	79	59.5	38±9.7		105±69	8.9	22.8	48.1	23±1.04	0
Zeng	2010	Cross-sectional	China	Low/middle	W/Pacific	82	39	38.4±6.4		405±191	50			34.7±17.1	7.3
All studies						83669	42.2	39.1±9.3	66.4±43.9	341±178	30.8	16.6	34.3	32.4±16.1	28

ART, antiretroviral therapy; F, females; N, total number of participants included in the analysis; PI, protease inhibitors; S/E Asia, South-East Asia; SSA, sub-Saharan Africa; UK, United Kingdom; USA, Unites States of America; W/Pacific, Western Pacific; Age, HIV duration, CD4 count and ART duration were reported as mean ± standard deviation

6.3 RISKS OF BIAS IN INCLUDED STUDIES

Table 6.2 presents the results of the risk of bias assessment for each included study. The risk of selection bias was low in 31 studies (34%). Exposure criterion was well-defined in all 91 studies. Outcome measurements were validated in 77 studies, indicating that the risk of information bias (with respect to the assessments of outcomes) was low in 85% of the included studies. Sixty-eight studies (75%) adjusted for one or more important confounders. Loss to follow-up was less than 20% in 23 of the 31 included cohort studies, suggesting a low risk of attrition bias in these studies.

Table 6.2: Results of risk of bias assessment for each included study

Study	Sampling (selection bias)	Exposure assessment (information bias)	Outcome assessment (information bias)	Adjust for confounders	Loss to follow-up (attrition bias)
Abebe 2014	high risk	low risk	low risk	low risk	not applicable
Abrahams 2015	high risk	low risk	low risk	low risk	low risk
Aboud 2010	low risk	low risk	low risk	low risk	not applicable
Arruda Junior 2010a	high risk	low risk	low risk	low risk	not applicable
Arruda Junior 2010b	unclear	low risk	low risk	low risk	not applicable
Adewole 2010	low risk	low risk	low risk	low risk	unclear
Akinboro 2013	unclear	low risk	low risk	low risk	not applicable
Awotedu 2010	high risk	low risk	low risk	low risk	not applicable
Ayodele 2013	unclear	low risk	low risk	low risk	not applicable
Baekken 2008	unclear	low risk	low risk	low risk	not applicable
Bajaj 2013	unclear	low risk	low risk	low risk	not applicable
Bergersen 2003	low risk	low risk	low risk	low risk	not applicable
Bergersen 2004	low risk	low risk	low risk	low risk	not applicable
Bergersen 2006	unclear	low risk	low risk	low risk	not applicable
Blass 2008	low risk	low risk	low risk	low risk	not applicable
Blumer 2008	low risk	low risk	low risk	low risk	low risk
Bonfati 2007	high risk	low risk	low risk	low risk	not applicable
Bonfati 2012	unclear	low risk	low risk	low risk	low risk
Buchacz 2008	low risk	low risk	low risk	low risk	unclear
Calza 2011	high risk	low risk	low risk	low risk	not applicable
Carey 2013	low risk	low risk	low risk	high risk	not applicable
Ceccato 2011	unclear	low risk	low risk	high risk	low risk
Chow 2003	high risk	low risk	unclear	low risk	low risk
Denue 2012	unclear	low risk	low risk	low risk	low risk
Denue 2013a	unclear	low risk	low risk	low risk	low risk
Denue 2013b	unclear	low risk	low risk	low risk	low risk
Dimodi 2014	unclear	low risk	low risk	low risk	not applicable
Domingos 2009	unclear	low risk	unclear	high risk	not applicable
Eira 2012	unclear	low risk	unclear	low risk	not applicable
Ekali 2013	unclear	low risk	low risk	low risk	not applicable
Esposito 2008	high risk	low risk	low risk	unclear	low risk
Fontas 2004	low risk	low risk	low risk	low risk	low risk
Friis-Møller 2003	low risk	low risk	low risk	low risk	low risk
Goedecke 2013	unclear	low risk	low risk	low risk	not applicable
Gowdaiah 2013	unclear	low risk	unclear	high risk	unclear
Grandominico 2008	unclear	low risk	low risk	low risk	high risk
Hansen 2009	unclear	low risk	unclear	low risk	not applicable
Howard 2014	low risk	low risk	low risk	low risk	not applicable
Jaff 2015	unclear	low risk	low risk	low risk	not applicable
Jain 2013	unclear	low risk	low risk	low risk	not applicable
Jantarapakde 2014	unclear	low risk	unclear	low risk	not applicable
Jerico 2005	unclear	low risk	low risk	low risk	not applicable
Kiage 2013	unclear	low risk	low risk	high risk	low risk
Kingsley 2008	unclear	low risk	low risk	low risk	not applicable

Study	Sampling (selection bias)	Exposure assessment (information bias)	Outcome assessment (information bias)	Adjust for confounders	Loss to follow-up (attrition bias)
Koethe 2015	low risk	low risk	low risk	low risk	low risk
Koppel 2000	unclear	low risk	low risk	low risk	not applicable
Lekakis 2009	unclear	low risk	low risk	low risk	not applicable
Levy 2005	low risk	low risk	low risk	low risk	low risk
Lin 2011	unclear	low risk	low risk	unclear	unclear
Magenta 2011	low risk	low risk	low risk	low risk	low risk
Malapati 2014	unclear	low risk	low risk	low risk	low risk
Maloberti 2013	unclear	low risk	low risk	low risk	not applicable
Manuthu 2008	unclear	low risk	unclear	low risk	not applicable
Mariz 2011	low risk	low risk	low risk	low risk	not applicable
Mbunkah 2014	unclear	low risk	low risk	low risk	not applicable
Medina-Torne 2012	unclear	low risk	low risk	unclear	not applicable
Mital 2013	unclear	low risk	unclear	high risk	not applicable
Mittal 2013	unclear	low risk	unclear	high risk	not applicable
Mohammed 2015	low risk	low risk	low risk	low risk	not applicable
Montes 2005	unclear	low risk	low risk	low risk	low risk
Muhammad 2013a	high risk	low risk	low risk	high risk	not applicable
Muhammad 2013b	high risk	low risk	low risk	high risk	not applicable
Mustapha 2011	unclear	low risk	low risk	high risk	not applicable
Ngala 2013	unclear	low risk	low risk	low risk	not applicable
Ngondi 2007	unclear	low risk	low risk	high risk	unclear
Ogundahunsi 2008	high risk	low risk	low risk	high risk	not applicable
Ogunmola 2014	unclear	low risk	low risk	high risk	not applicable
Owiredu 2011	unclear	low risk	low risk	low risk	not applicable
Palacios 2006	unclear	low risk	low risk	low risk	low risk
Peck 2014	low risk	low risk	low risk	low risk	not applicable
Pefura Yone 2011	low risk	low risk	low risk	high risk	not applicable
Samaras 2007	unclear	low risk	low risk	low risk	not applicable
Shahmanesh 2004	high risk	low risk	low risk	low risk	not applicable
Silva 2009	high risk	low risk	low risk	low risk	not applicable
Silva 2010	unclear	low risk	unclear	high risk	not applicable
Singh 2014	unclear	low risk	low risk	high risk	low risk
Smith 2004	low risk	low risk	high risk	low risk	not applicable
Scholten 2011	unclear	low risk	low risk	low risk	not applicable
Shapiro 2012	high	low risk	low risk	low risk	not applicable
Soares 2015	high risk	low risk	low risk	high risk	high risk
Søgaard 2010	low risk	low risk	low risk	low risk	low risk
Sreekantamurthy 2014	low risk	low risk	unclear	high risk	not applicable
Tadewos 2012	low risk	low risk	low risk	low risk	not applicable
Tesfaye 2014	low risk	low risk	low risk	low risk	not applicable
Thiebaut 2005	low risk	low risk	low risk	low risk	low risk
van Leth 2004	low risk	low risk	low risk	low risk	high risk
Wanke 2005	high risk	low risk	low risk	low risk	low risk
Weerakkody 2013	low risk	low risk	unclear	high risk	not applicable
Wilson 2009	low risk	low risk	low risk	low risk	not applicable
Zannou 2009	low risk	low risk	unclear	low risk	low risk
Zeng 2010	unclear	low risk	low risk	high risk	not applicable

CHAPTER SEVEN

RESULTS – THE IMPACT OF ANTIRETROVIRAL THERAPY ON BLOOD PRESSURE AND HYPERTENSION RISK

In this chapter, I present meta-analyses of the associations of antiretroviral therapy with blood pressure and hypertension risk. I also identify study-level factors that may influence these pooled associations.

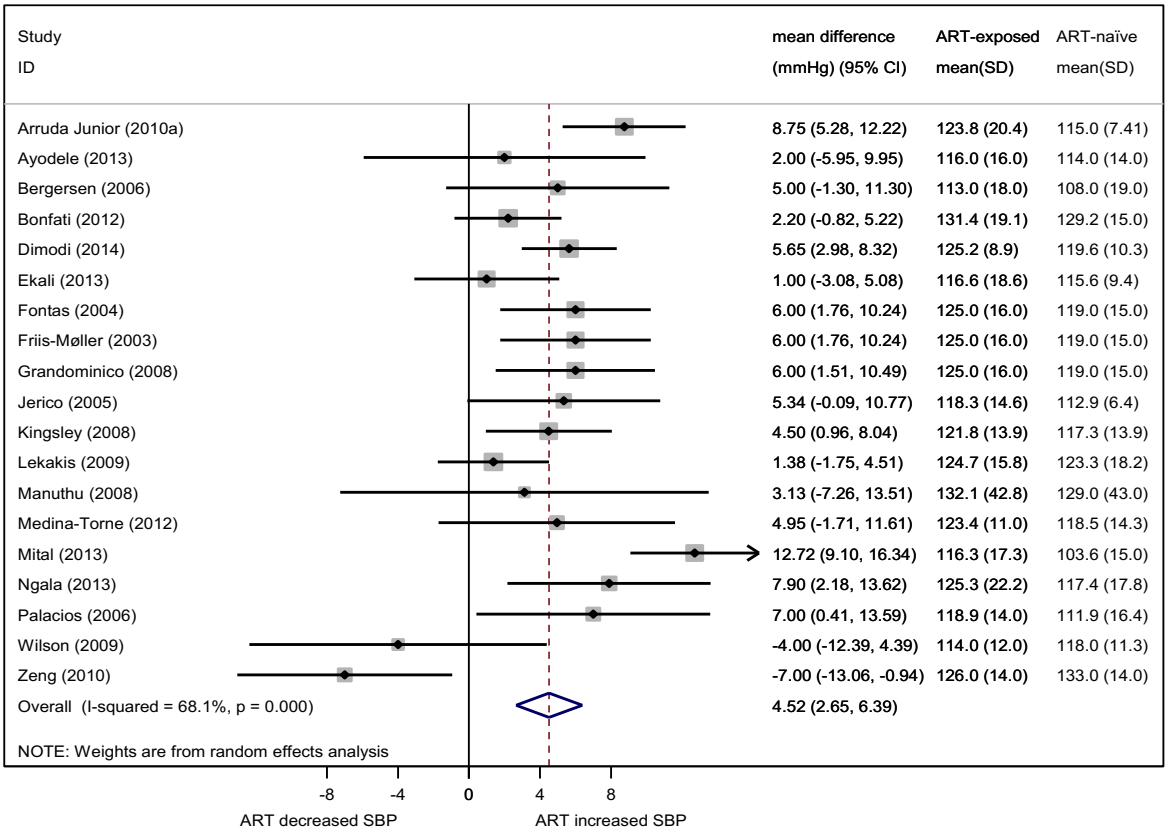
7.1 OVERALL ASSOCIATIONS OF ANTIRETROVIRAL THERAPY WITH BLOOD PRESSURE AND HYPERTENSION RISK

7.1.1 Increase in systolic blood pressure

Nineteen studies compared systolic blood pressure changes between patients naïve and exposed to antiretroviral therapy, nine of which reported significantly higher mean systolic blood pressure in antiretroviral-exposed patients compared with antiretroviral naïve patients (Arruda Junior *et al*., 2010a; Dimodi *et al*., 2014; Fontas *et al*., 2004; Grandominico *et al*., 2008; Kingsley *et al*., 2008; Mital *et al*., 2013; Ngala *et al*., 2013; Palacios *et al*., 2006) (Figure 7.1). Mean systolic blood pressure was also higher in eight of the remaining ten studies ― albeit non-significantly (Ayodele *et al*., 2013; Bergersen *et al*., 2006; Bonfati *et al*., 2012; Ekali *et al*., 2013; Lekakis *et al*., 2009; Manuthu *et al*., 2008; Medina-Torne *et al*., 2012; Jerico *et al*., 2005), leaving only two small-sized studies with higher systolic blood pressure in antiretroviral-naïve patients, compared to patients exposed to antiretroviral therapy (Wilson *et al*., 2009; Zeng *et al*., 2010). Overall, mean systolic blood pressure was significantly higher among patients exposed to antiretroviral therapy compared with treatment-naïve patients (Pooled MD 4.52 mmHg, 95% CI 2.65 to 6.39). Heterogeneity across the studies included in the meta-analysis was statistically

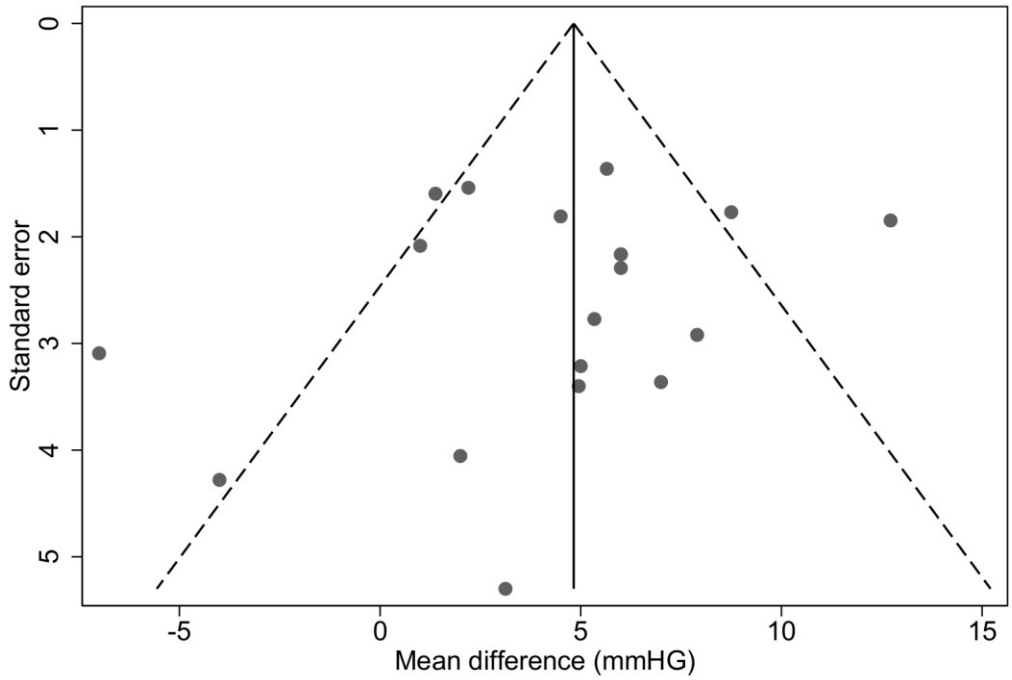
significant ($P < 0.001$ for Chi^2 test for heterogeneity), and accounted for a moderate amount of the variability in the effect estimates between these studies ($I^2 = 68\%$). Funnel plot asymmetry was absent, suggesting no evidence of publication bias in the observed association between antiretroviral therapy and increased systolic blood pressure ($P = 0.43$ for Egger's regression test for funnel plot asymmetry) (Figure 7.2). Sensitivity analysis revealed no instance in which the exclusion of a study altered the interpretation of the pooled association (Table 7.1).

Figure 7.1: Meta-analysis of the association between antiretroviral therapy and systolic blood pressure



ART, antiretroviral therapy; CI, confidence interval; SBP, systolic blood pressure. The forest plot above shows 19 black circles plotted on 19 horizontal lines, which correspond to the 19 studies included in the meta-analysis. Each black circle represents the mean difference in systolic blood pressure between antiretroviral-exposed and antiretroviral-naïve patients for each included study. The ends of each horizontal line correspond to the 95% confidece intervals of each mean difference. The diamond at the bottom of the graph shows the pooled mean difference in SBP for all 19 studies: the dashed vertical line through the diamond represents the pooled WMD, whereas the two lateral vertices of the diamond represent the 95% confidence intervals of the pooled WMD. The solid vertical line through zero represents no effect.

Figure 7.2: Analysis of publication bias regarding association between exposure to antiretroviral therapy and mean systolic blood pressure

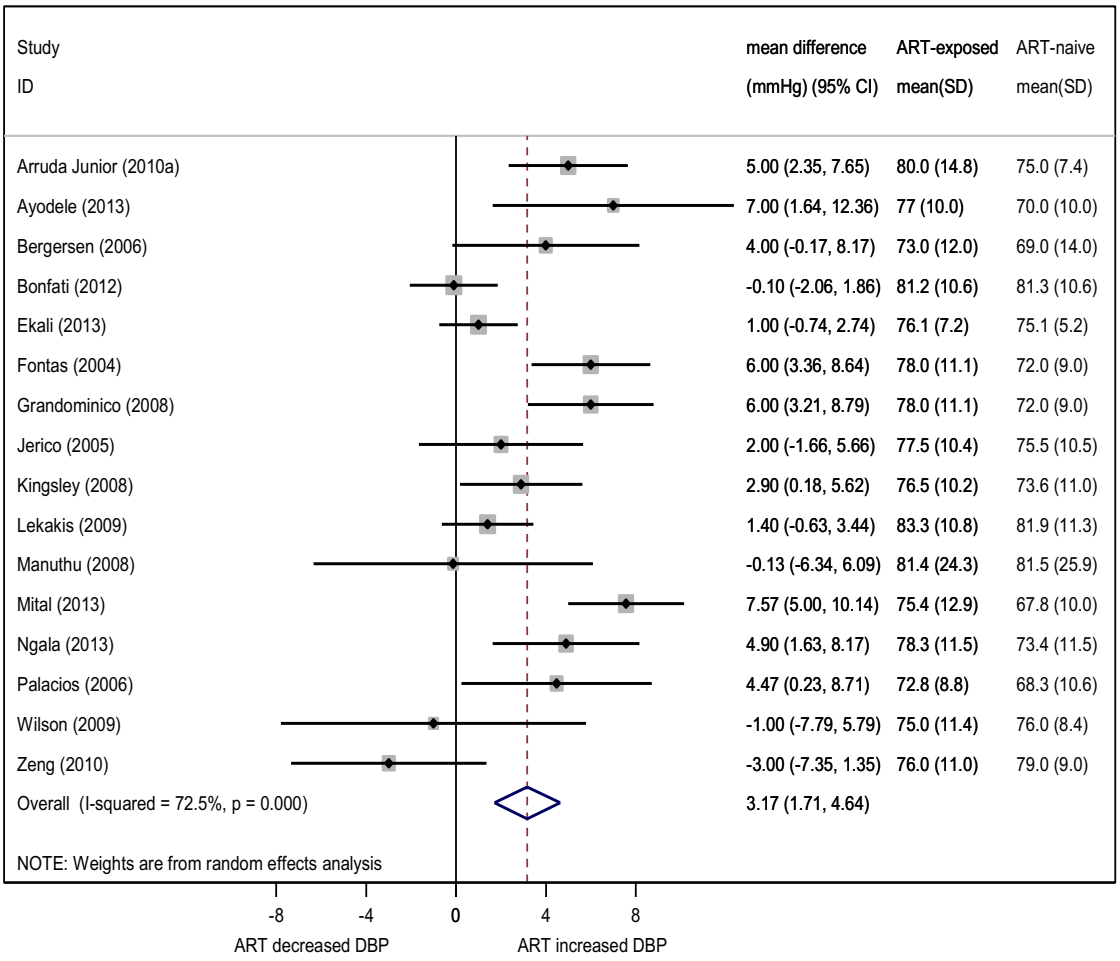


Funnel plot showing mean difference in systolic blood pressure for each of the 19 included studies plotted against the standard errors. The 19 circles correspond to the 19 studies included meta-analysis. The solid vertical line represents the pooled effect of antiretroviral therapy on systolic blood pressure. The two interrupted diagonal lines represent the 95% confidence intervals around the pooled effect. The smaller studies represented by circles towards the bottom of the graph are not biased towards a larger effect of antiretroviral therapy on systolic blood pressure. $P = 0.43$ for Egger's test for small study effects.

7.1.2 Increase in diastolic blood pressure

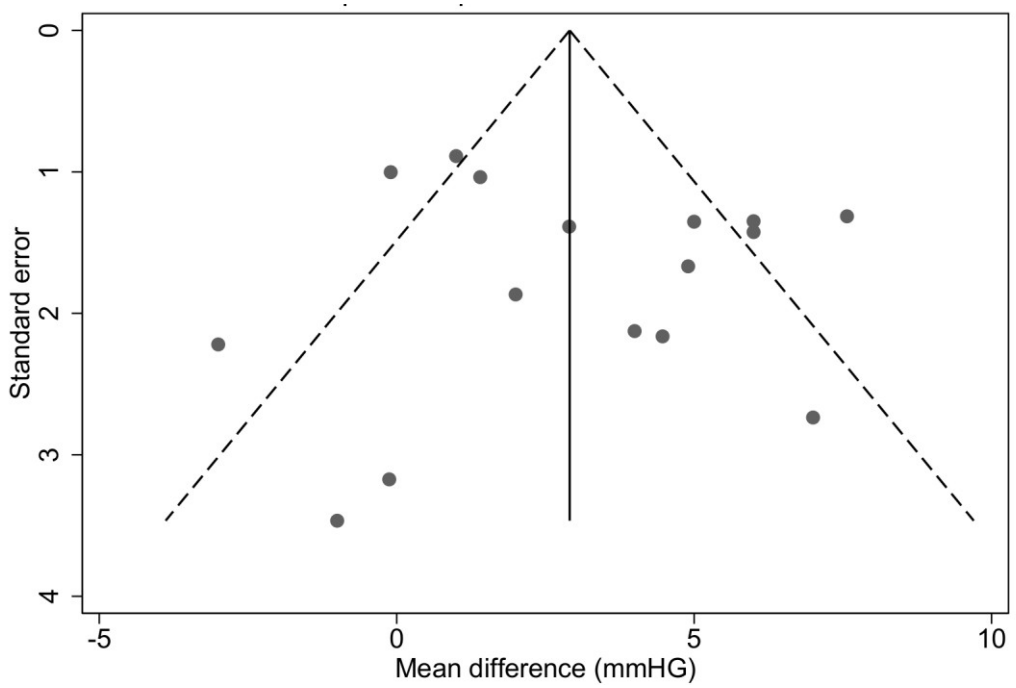
Eight of 16 studies reported significantly higher mean diastolic blood pressure in patients exposed to antiretroviral therapy, compared to antiretroviral-naïve patients (Arruda Junior *et al*., 2010a; Ayodele *et al*., 2013; Fontas *et al*., 2004; Grandominico *et al*., 2008; Kingsley *et al*., 2008; Mital *et al*., 2013; Ngala *et al*., 2013; Palacios *et al*., 2006) (Figure 7.3). Four of the other eight studies reported non-significant increases in mean diastolic pressure among antiretroviral-exposed patients compared to antiretroviral-naïve patients (Bergersen *et al*., 2006; Ekali *et al*., 2013; Jerico *et al*., 2005; Lekakis *et al*., 2009), leaving four studies with higher mean diastolic blood pressure measurements in antiretroviral-naïve compared to antiretroviral-exposed patients (Bonfati *et al*., 2012; Manuthu *et al*., 2008; Wilson *et al*., 2009; Zeng *et al*., 2010). However, the mean diastolic blood pressure for patients exposed to antiretroviral therapy was significantly higher, compared to antiretroviral-naïve patients, overall (Pooled MD 3.17 mmHg, 95% CI 1.71 to 4.64). Heterogeneity across the included studies was statistically significant ($P < 0.001$ for Chi^2 test for heterogeneity), and accounted a substantial amount of between-study variability in the effect estimates ($I^2 = 72.5\%$). There was also no evidence of funnel plot asymmetry or publication bias ($P = 0.56$ for Egger's regression test for asymmetry) (Figure 7.4). Sensitivity analysis revealed no instance in which the exclusion of a study altered the interpretation of the pooled association (Table 7.1).

Figure 7.3: Meta-analysis of the association between antiretroviral therapy and diastolic blood pressure



ART, antiretroviral therapy; CI, confidence interval; DBP, diastolic blood pressure. The forest plot above shows 16 black circles plotted on 16 horizontal lines, which correspond to the 16 studies included in the meta-analysis. Each black circle represents the mean difference in diastolic blood pressure between antiretroviral-exposed and antiretroviral-naïve patients for each included study. The ends of each horizontal line correspond to the 95% confidece intervals of each mean difference. The diamond at the bottom of the graph shows the pooled mean difference in DBP for all 16 studies: the dashed vertical line through the diamond represents the pooled mean difference, whereas the two lateral vertices of the diamond represent the 95% confidence intervals of the pooled mean difference. The solid vertical line through zero represents no effect.

Figure 7.4: Analysis of publication bias regarding association between exposure to antiretroviral therapy and mean diastolic blood pressure

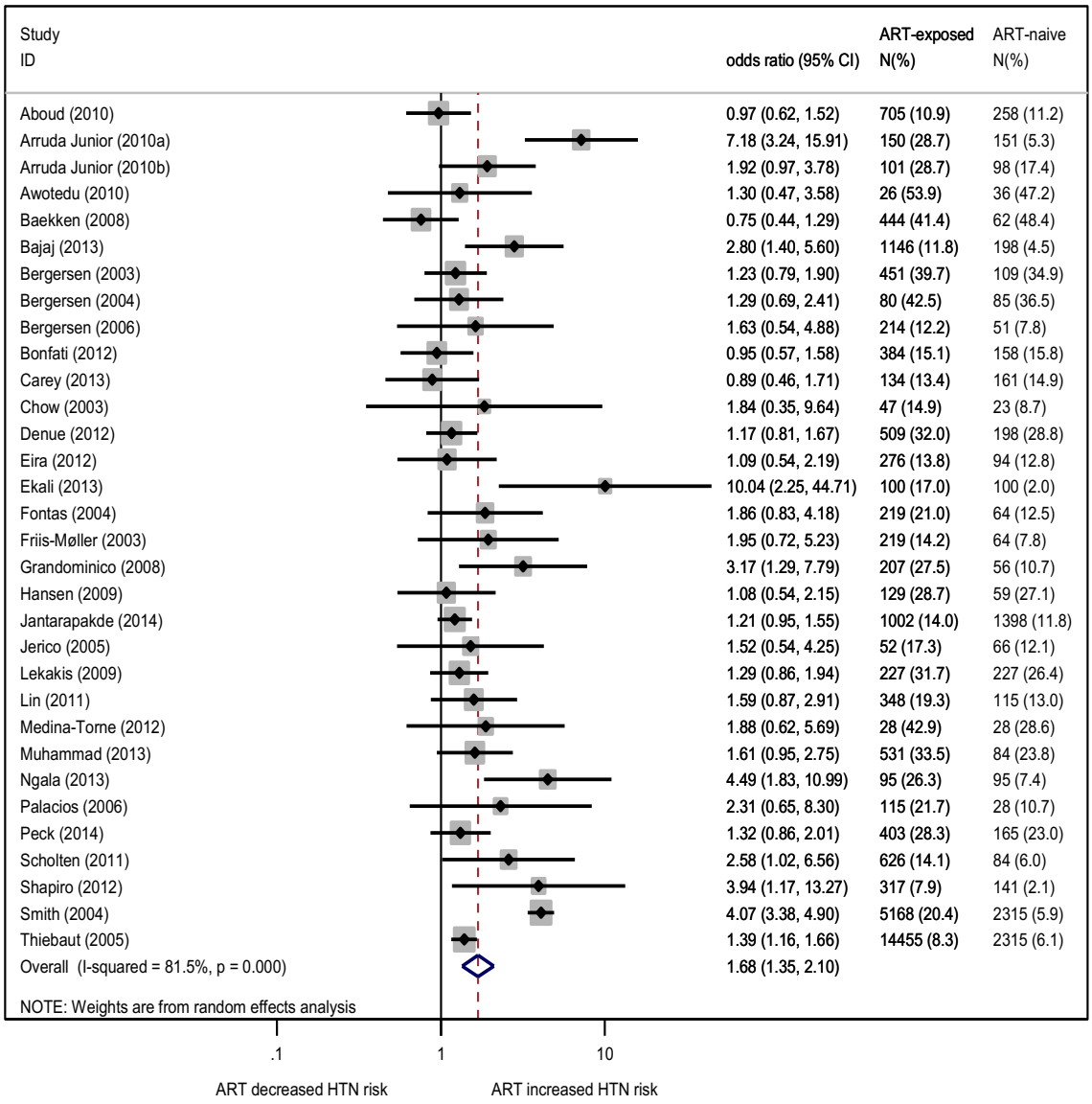


Funnel plot showing mean difference in diastolic blood pressure for each of the 16 included studies plotted against the standard errors. The 16 circles correspond to the 16 studies included meta-analysis. The solid vertical line represents the pooled effect of antiretroviral therapy on diastolic blood pressure. The two interrupted diagonal lines represent the 95% confidence intervals around the pooled effect. The smaller studies represented by circles towards the bottom of the graph are not biased towards a larger effect of antiretroviral therapy on diastolic blood pressure. $P = 0.56$ for Egger's test for small study effects.

7.1.3 Increase in hypertension risk

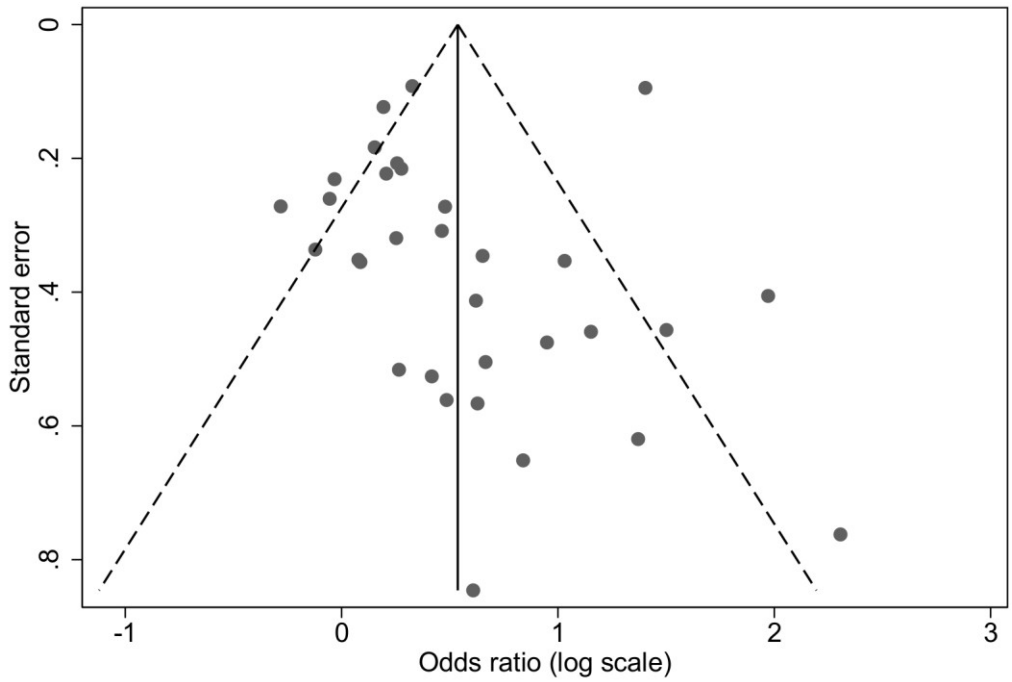
Prevalence estimates of hypertension were compared between antiretroviral-exposed and antiretroviral-naïve HIV-infected patients in 32 studies, 28 of which reported higher estimates in the antiretroviral-exposed group. Overall, hypertension was reported in 4,195 of 28,908 (14.5%) antiretroviral-exposed patients, as opposed to 950 of 9,086 (10.5%) antiretroviral-naïve patients, such that the odds of hypertension was significantly higher in antiretroviral-exposed patients (Pooled OR 1.68, 95% CI 1.35 to 2.10; $P < 0.001$) (Figure 7.5). Heterogeneity across the 32 studies was statistically significant ($P < 0.001$ for Chi^2 test for heterogeneity) and accounted for a considerable amount of between-study variability in the effect estimates ($I^2 = 81.5\%$). Although funnel plot revealed a rather high tendency for smaller studies to report larger effects of antiretroviral therapy on hypertension risk ($P = 0.006$ for Egger's regression test for funnel plot asymmetry) (Figure 7.6), correction for publication bias using the trim and fill analysis did not alter the overall interpretation of the pooled association. The trim and fill analysis imputed 12 'missing' studies to the original dataset; however, the pooled estimate for the trimmed and filled dataset remained statistically significant ($P < 0.001$). The variance between studies (0.000) and heterogeneity ($P = 1.000$) remained the same in both original and filled datasets. The funnel plot for the filled dataset was approximately symmetrical (Figure 7.7). Sensitivity analysis revealed no instance in which the sequential exclusion of studies altered the interpretation of the pooled association (Table 7.1).

Figure 7.5: Meta-analysis of the association between antiretroviral therapy and hypertension risk



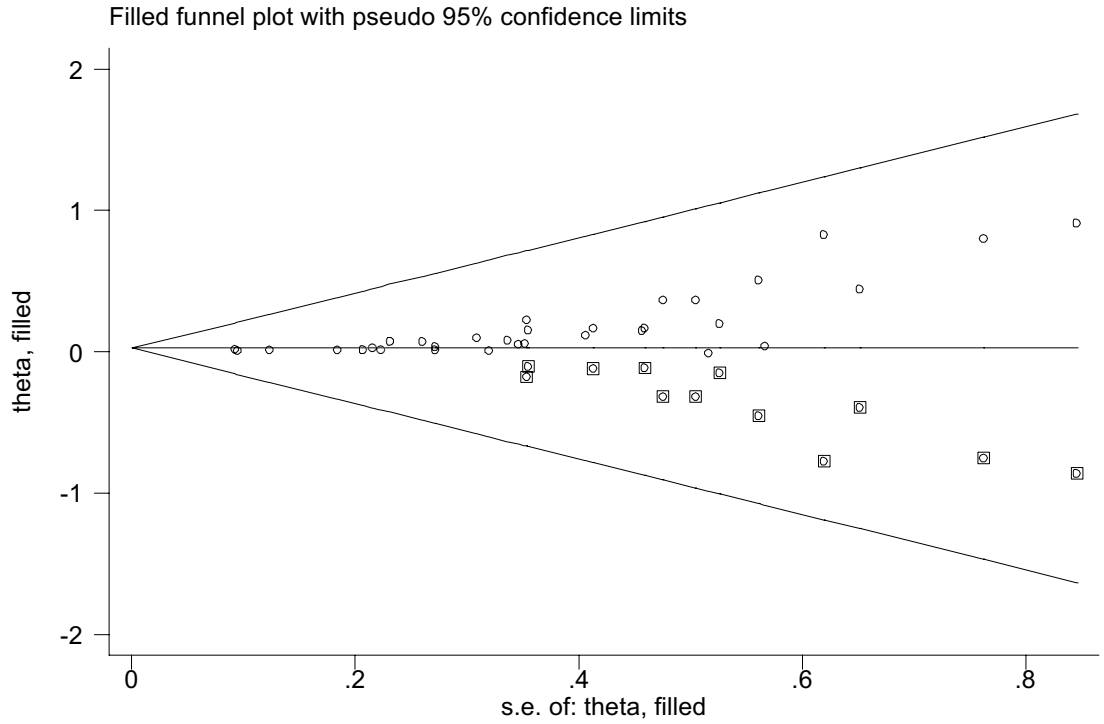
ART, antiretroviral therapy; CI, confidence interval; HTN, hypertension; OR, odds ratio. The black circles and horizontal lines represent the OR with 95% CI for hypertension in each study. The dashed vertical line through the diamond represents the pooled OR, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled OR. The solid vertical line through one represents no effect.

Figure 7.6: Analysis of publication bias in the association between antiretroviral therapy and hypertension



Funnel plot showing odds ratios of hypertension for each of the 32 included studies plotted against the standard errors. The 32 circles correspond to the 32 studies included in the meta-analysis. The smaller studies represented by circles towards the bottom of the graph appear biased towards larger odds ratios.
P = 0.006 for Egger's test for small study effects.

Figure 7.7: Filled funnel plot for analysis of trimmed and filled dataset for the association between antiretroviral therapy and hypertension risk



Symmetrical funnel plot showing the final filled effect estimate (represented by the horizontal line) and the imputed data (represented by the 12 squares around the circular data symbols). The diagonal lines represent the 95% confidence intervals around the final filled estimate. Theta stands for the log scale of the effect estimate.

Table 7.1: Sensitivity analysis of the pooled associations of antiretroviral therapy with increased blood pressure and hypertension risk

Cardio-metabolic outcomes	Pooled estimate prior to serial exclusion of individual studies Pooled ES (95% CI)	Range of pooled estimate following serial exclusion of individual studies	Instance in which exclusion of a study changed interpretation of the pooled association Pooled ES (95% CI)
Systolic blood pressure	4.52 (2.65 to 6.39)	4.22 to 4.74	None
Diastolic blood pressure	3.17 (1.71 to 4.64)	3.01 to 3.47	None
Hypertension	1.68 (1.35 to 2.10)	1.61 to 1.73	None

CI, confidence interval; Pooled ES, Pooled estimate.

7.2. FACTORS THAT MAY INFLUENCE THE ASSOCIATIONS OF ANTIRETROVIRAL THERAPY WITH INCREASED BLOOD PRESSURE AND HYPERTENSION RISK

Table 7.2 presents the results of subgroup and meta-regression analyses of the association between antiretroviral therapy and systolic blood pressure. Antiretroviral therapy was significantly associated with higher systolic blood pressure levels across subgroups of different study-level characteristics, including country income group, sex, smoking status, HIV infection duration, study design, year of publication and risk of selection bias. In addition, no statistically significant difference in the pooled estimate was observed between subgroups of these characteristics. With the exception of geographical region, antiretroviral therapy was also associated with higher systolic blood pressure levels across other study-level characteristics. However, these associations were not significant across subgroups of these characteristics. For instance, antiretroviral therapy was significantly associated with increased systolic blood pressure among HIV-infected patients who were 40 years of age and older (Pooled MD 4.88 mmHg, 95% CI 3.01 to 6.76), but not among HIV-infected patients who were younger than 40 years (Pooled MD 3.41 mmHg, 95% CI -0.93 to 7.75). Nonetheless, the difference between both subgroup estimates was not statistically significant (P = 0.565 for interaction). Similarly, antiretroviral therapy was significantly associated with increased systolic blood pressure among HIV-infected patients with CD4 cell counts below 350 cells/mm^3 (Pooled MD 4.28 mmHg, 95% CI 1.86 to 6.71), but in those with greater CD4 cell counts (Pooled MD 2.98 mmHg, 95% CI -0.17 to 6.13) (P = 0.547 for interaction). Antiretroviral therapy was also significantly associated with increased systolic blood pressure among HIV-infected patients on treatment for 18 months or longer (Pooled MD 4.03 mmHg, 95% CI 1.59 to 6.48), but not among patients with antiretroviral treatment duration of less than 18 months (Pooled

MD 6.13 mmHg, 95% CI -0.25 to 12.52) (P = 0.383 for interaction). Although subgroup analysis by geographical region revealed a significant association between antiretroviral therapy and decreased systolic blood pressure (MD -7.00, 95% CI -13.06 to -0.94) among patients in the Western Pacific region, it is worth emphasizing that this finding was solely based on one study (Zang *et al*., 2010).

Table 7.2: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and systolic blood pressure

			Meta-regression	
Subgroups	**N**	**Pooled MD (95% CI)**	***P* value**	**R^2 (%)**
Region				
Sub-Saharan Africa	5	7.90 (2.18 to 13.62)		
Europe	8	3.69 (1.75 to 5.63)		
The Americas	4	6.36 (4.26 to 8.46)		
South-East Asia	1	12.72 (9.10 to 16.34)		
Western Pacific	1	-7.00 (-13.06 to -0.94)	0.865	0
Income group				
High income	11	3.98 (2.55 to 5.42)		
Low/middle income	8	4.69 (0.74 to 8.65)	0.703	0
Females				
< 50%	11	3.05 (0.82 to 5.28)		
≥ 50%	6	6.80 (3.05 to 10.54)	0.089	18.9
Age				
< 40 years	8	3.41 (-0.93 to 7.75)		
≥ 40 years	10	4.88 (3.01 to 6.76)	0.565	0
Smokers				
< 50%	8	3.05 (0.46 to 5.65)		
≥ 50%	7	5.24 (3.12 to 7.37)	0.413	0
CD4 count				
< 350 cells/mm^3	9	4.28 (1.86 to 6.71)		
≥ 350 cells/mm^3	7	2.98 (-0.17 to 6.13)	0.547	0
HIV duration				
< 60 months	5	4.36 (1.93 to 6.80)		
≥ 60 months	7	3.88 (2.22 to 5.54)	0.847	0
ART duration				
< 18 months	4	6.13 (-0.25 to 12.52)		
≥ 18 months	11	4.03 (1.59 to 6.48)	0.383	4.6
Study design				
Cohort	4	4.55 (2.21 to 6.89)		
Cross-sectional	15	4.29 (1.94 to 6.64)	0.768	0
Selection bias				
Low risk	8	3.91 (2.48 to 5.32)		
High/unclear risk	11	4.30 (1.01 to 7.59)	0.782	0
Publication year				
2000 – 2009	10	4.29 (2.71 to 5.88)		
2010 – 2015	9	4.54 (1.17 to 7.91)	0.867	0

ART, antiretroviral therapy; CI, confidence interval; MD, mean difference, N, number of studies; R^2, amount of explained variability

Antiretroviral therapy was significantly associated with increased diastolic blood pressure across subgroups of different study-level characteristics including country income group, sex, age, smoking status, antiretroviral treatment duration, risk of selection bias and year of publication (Table 7.3). With the exception of smoking status, no statistically significant difference in the pooled estimate was observed between subgroups of these characteristics. Estimates of the pooled association of antiretroviral therapy with increased diastolic blood pressure were significantly higher in studies with more smokers than non-smokers (Pooled MD 5.46 mmHg, 95% CI 3.86 to 7.06), compared to studies with fewer smokers than non-smokers (Pooled MD 1.65 mmHg, 95% CI 0.08 to 3.23) (P = 0.025 for interaction). Meta-regression also revealed that study-level differences in the proportions of smokers accounted approximately for 67% of the variability between studies included in the meta-analysis.

Mean diastolic blood pressure levels were also higher among antiretroviral-exposed patients, compared to antiretroviral-naïve patients across subgroups of CD4 cell count, HIV infection duration and study design. However, some of these associations were not statistically significant. For instance, antiretroviral therapy was significantly associated with increased diastolic blood pressure among HIV-infected patients with CD4 cell counts below 350 cells/mm^3 (Pooled MD 2.82 mmHg, 95% CI 1.46 to 4.18), but not among HIV-infected patients with CD4 cell counts greater than 350 cells/mm^3 (Pooled MD 2.73 mmHg, 95% CI -0.58 to 6.05). Nonetheless, the difference between both subgroup estimates was not statistically significant (P = 0.928 for interaction). Similarly, antiretroviral therapy was significantly associated with increased diastolic blood pressure among patients who had been living with HIV for less than 60 months (Pooled MD 3.22 mmHg, 95% CI 0.89 to 5.56), but not among patients who had been infected for 60 months or longer (Pooled MD 2.71 mmHg, 95% CI -0.05 to 5.47) (P = 0.733 for

interaction). Unlike cohort studies (Pooled MD 3.31 mmHg, 95% CI -1.00 to 7.62), cross-sectional studies were likely to report a significant association between antiretroviral therapy and increased diastolic blood pressure (Pooled MD 3.18 mmHg, 95% CI 1.56 to 4.79), however, the difference between both subgroup estimates was not statistically significant ($P = 0.983$ for interaction).

Table 7.3: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and diastolic blood pressure

			Meta-regression	
Subgroups	**N**	**Pooled MD (95% CI)**	***P* value**	**R^2 (%)**
Region				
Sub-Saharan Africa	4	3.04 (0.02 to 6.07)		
Europe	7	2.49 (0.51 to 4.46)		
The Americas	3	4.62 (2.85 to 6.39)		
South-East Asia	1	7.57 (5.00 to 10.15)		
Western Pacific	1	-3.00 (-7.35 to 1.35)	1.000	0
Income group				
High income	9	2.98 (1.26 to 4.69)		
Low/middle income	7	3.37 (0.62 to 6.13)	0.779	0
Females				
< 50%	10	2.64 (0.67 to 4.60)		
≥ 50%	6	3.98 (1.56 to 6.36)	0.403	0
Age				
< 40 years	8	2.55 (0.27 to 4.83)		
≥ 40 years	8	3.73 (1.66 to 5.79)	0.460	0
Smokers				
< 50%	7	1.65 (0.08 to 3.23)		
≥ 50%	5	5.46 (3.86 to 7.06)	0.025	66.9
CD4 count				
< 350 cells/mm^3	8	2.82 (1.46 to 4.18)		
≥ 350 cells/mm^3	6	2.73 (-0.58 to 6.05)	0.928	0
HIV duration				
< 60 months	4	3.22 (0.89 to 5.56)		
≥ 60 months	5	2.71 (-0.05 to 5.47)	0.733	0
ART duration				
< 18 months	4	4.85 (2.01 to 7.69)		
≥ 18 months	9	2.80 (0.97 to 4.64)	0.309	8.9
Study design				
Cohort	3	3.31 (-1.00 to 7.62)		
Cross-sectional	13	3.18 (1.56 to 4.79)	0.983	0
Sampling bias				
Low risk	2	2.91 (0.09 to 5.61)		
High/unclear risk	14	3.17 (1.62 to 4.76)	0.760	0
Publication year				
2000 – 2009	9	4.29 (2.71 to 5.88)		
2010 – 2015	7	3.36 (1.81 to 4.90)	0.926	0

ART, antiretroviral therapy; CI, confidence interval; MD, mean difference, N, number of studies; R^2, amount of explained variability.

With the exception of geographical region and antiretroviral treatment duration, antiretroviral therapy was significantly associated with increased risk of hypertension across the subgroups of all study-level characteristics, with no evidence of a statistically significant difference between subgroup estimates of the pooled association (Table 7.4). The odds of hypertension were significantly higher among antiretroviral-exposed patients who had been on treatment for no less than 18 months, compared to antiretroviral-naïve patients (Pooled OR 2.23, 95% CI 1.55 to 3.20), whereas the odds of hypertension were not statistically significantly different between HIV-infected patients exposed to antiretroviral therapy for less than 18 months and HIV-infected patients who were naïve to antiretroviral therapy (Pooled OR 1.81, 95% CI 0.65 to 5.04). However, meta-regression analysis revealed no statistically significant difference between both subgroup estimates (P = 0.603 for interaction). Similarly, while antiretroviral therapy was significantly associated with increased risk of hypertension among HIV-infected patients resident in sub-Saharan African countries (Pooled OR 1.99, 95% CI 1.33 to 2.98) and the Americas (Pooled OR 2.37, 95% CI 1.30 to 4.31), no statistically significant association was observed among HIV-infected patients resident in Europe (Pooled OR 1.42, 95% CI 0.99 to 2.05), South-East Asia (Pooled OR 1.39, 95% CI 0.81 to 2.39), and the Western Pacific region (OR 1.59, 95% CI 0.87 to 2.91) (P = 0.865 for interaction).

Table 7.4: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and hypertension risk

			Meta-regression	
Subgroups	**N**	**Pooled OR (95% CI)**	***P* value**	**R^2 (%)**
Region				
Sub-Saharan Africa	8	1.99 (1.33 to 2.98)		
Europe	14	1.42 (0.99 to 2.05)		
The Americas	6	2.37 (1.30 to 4.31)		
South-East Asia	3	1.39 (0.81 to 2.39)		
Western Pacific	1	1.59 (0.87 to 2.91)	0.865	0
Income group				
High income	18	1.52 (1.10 to 2.09)		
Low/middle income	14	1.89 (1.40 to 2.56)	0.767	0
Females				
< 50%	17	1.61 (1.16 to 2.24)		
≥ 50%	11	1.83 (1.26 to 2.65)	0.636	0
Age				
< 40 years	14	1.60 (1.07 to 2.39)		
≥ 40 years	10	2.03 (1.36 to 3.02)	0.739	0
Smokers				
< 50%	14	1.67 (1.09 to 2.57)		
≥ 50%	7	2.18 (1.61 to 2.97)	0.558	0
CD4 count				
< 350 cells/mm^3	7	2.96 (1.53 to 5.72)		
≥ 350 cells/mm^3	11	1.73 (1.12 to 2.69)	0.586	0
HIV duration				
< 60 months	4	1.64 (1.04 to 2.58)		
≥ 60 months	9	2.01 (1.30 to 3.01)	0.733	0
ART duration				
< 18 months	3	1.81 (0.65 to 5.04)		
≥ 18 months	15	2.23 (1.55 to 3.20)	0.603	0
Study design				
Cohort	8	1.37 (1.15 to 1.63)		
Cross-sectional	24	1.75 (1.31 to 2.33)	0.971	0
Selection bias				
Low risk	8	1.52 (1.14 to 2.04)		
High/unclear risk	24	1.65 (1.27 to 2.24)	0.520	0
Publication year				
2000 – 2009	14	1.62 (1.11 to 2.36)		
2010 – 2015	18	1.68 (1.31 to 2.14)	0.705	0

ART, antiretroviral therapy; CI, confidence interval; N, number of studies; OR, odds ratio; R^2, amount of explained variability

CHAPTER EIGHT

RESULTS – THE IMPACT OF ANTIRETROVIRAL THERAPY ON SERUM LIPOPROTEIN LEVELS AND DYSLIPIDAEMIAS

In this chapter, I present meta-analyses of the associations of antiretroviral therapy with serum lipid levels. I also identify study-level factors that may influence these pooled associations.

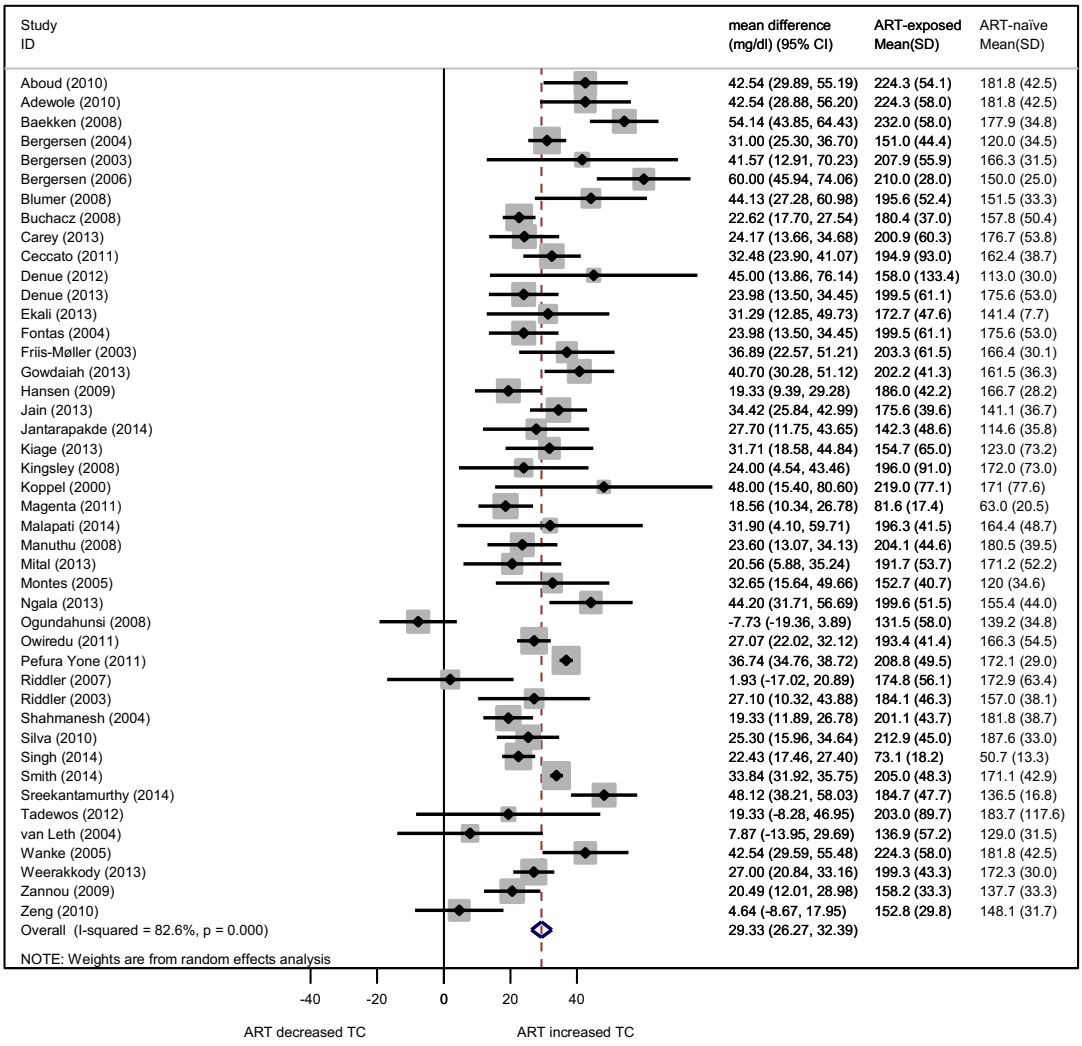
8.1 OVERALL ASSOCIATIONS OF ANTIRETROVIRAL THERAPY WITH SERUM LIPOPROTEIN LEVELS AND DYSLIPIDAEMIAS

8.1.1 Increase in serum levels of total cholesterol

Serum total cholesterol concentrations were compared between patients naïve and exposed to antiretroviral therapy in 45 studies, 40 of which reported significantly higher mean concentrations in antiretroviral-exposed persons (Figure 8.1). Mean total cholesterol levels were also higher — albeit statistically non-significantly — among antiretroviral-exposed patients, compared to their naïve counterparts in four of the five remaining studies. The difference was not statistically significant in the one study that reported higher mean total cholesterol in antiretroviral-naïve patients compared with antiretroviral-exposed patients (Adewole *et al*., 2010). Overall, the mean total cholesterol level was significantly higher among HIV-infected patients on antiretroviral therapy compared with treatment-naïve patients (Pooled MD 29.33 mg/dL, 95% CI 26.27 to 32.35). Heterogeneity was statistically significant across all 45 studies included in the meta-analysis ($P < 0.001$ for Chi^2 test for heterogeneity). I^2 statistic was 82.6%, indicating that heterogeneity across the studies accounted for a considerable amount of the variability in the effect estimates obtained from each study. There was no evidence of

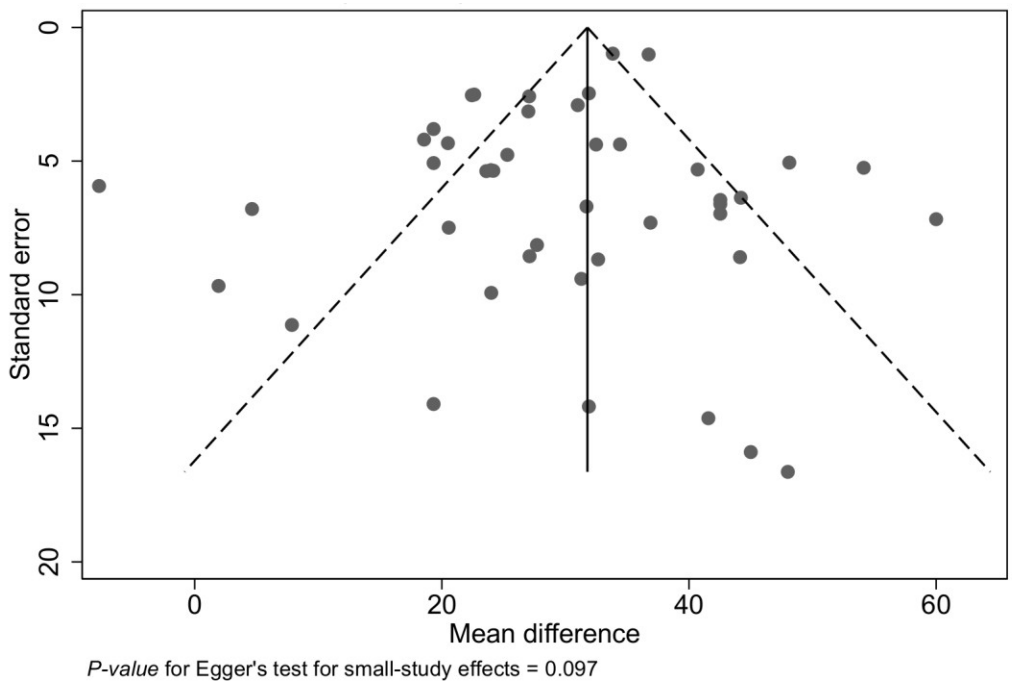
publication bias, as shown by the absence of funnel plot asymmetry (Figure 8.2) and Egger's regression test for funnel plot asymmetry (p= 0.097). Sensitivity analysis revealed no instance in which the sequential exclusion of studies altered the interpretation of the pooled association (Table 8.1).

Figure 8.1: Meta-analysis of the association between antiretroviral therapy and total cholesterol



ART, antiretroviral therapy; CI, confidence interval; TC, total cholesterol. The black boxes and horizontal lines represent the mean difference in TC with 95% CI in each study. The dashed vertical line through the diamond represents the pooled mean difference, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled mean difference. The solid vertical line through zero represents no effect.

Figure 8.2: Analysis of publication bias regarding association between antiretroviral therapy and total cholesterol level

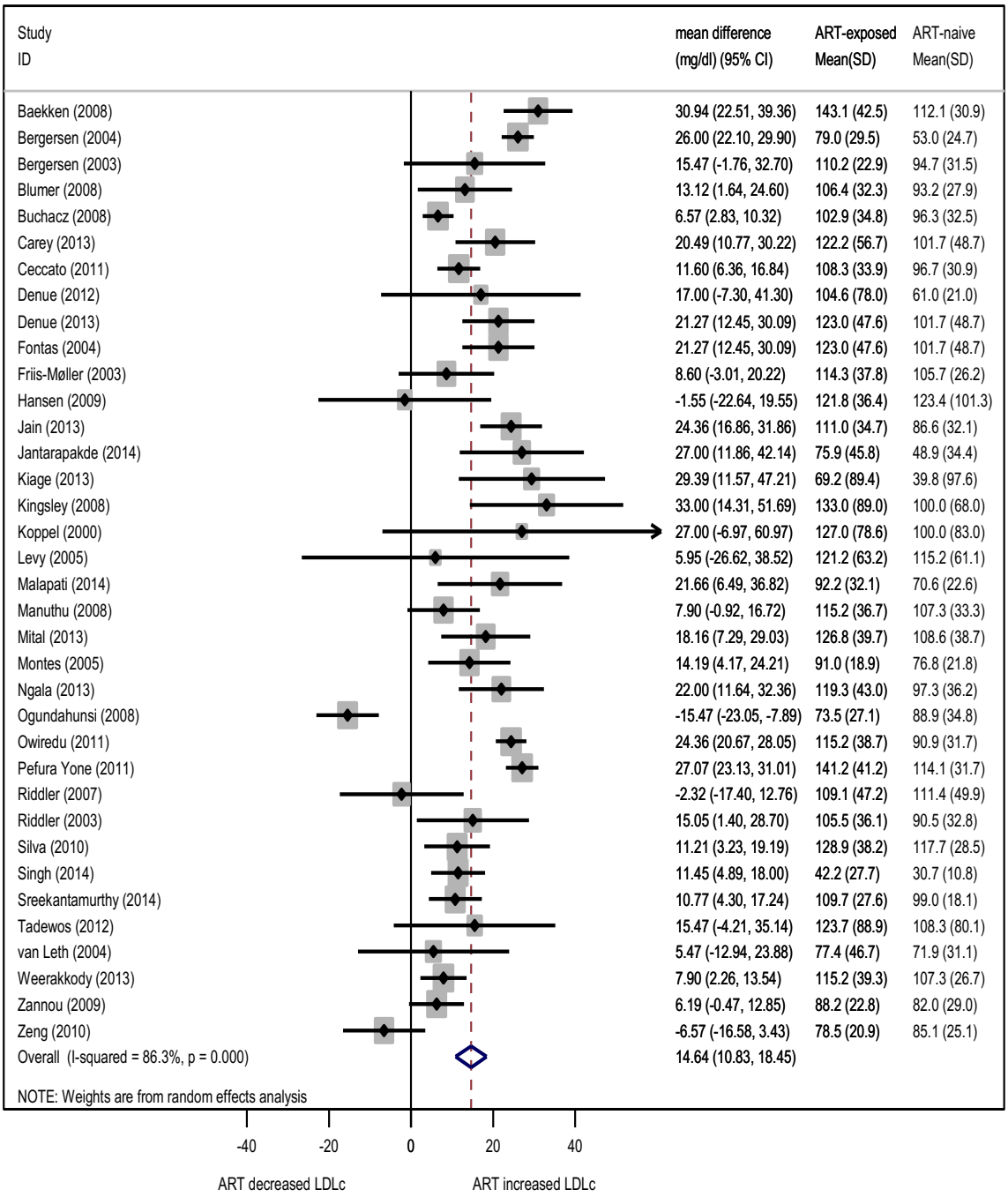


Funnel plot showing mean difference in total cholesterol for each of the 45 included studies plotted against the standard errors. The 45 circles correspond to the 45 studies included in the meta-analysis. The solid vertical line represents the pooled effect of antiretroviral therapy on total cholesterol. The two dashed lines represent the 95% confidence intervals around the pooled effect. The smaller studies represented by circles towards the bottom of the graph are not biased towards a larger effect of antiretroviral therapy on total cholesterol.

8.1.2 Increase in serum levels of low density lipoprotein cholesterol

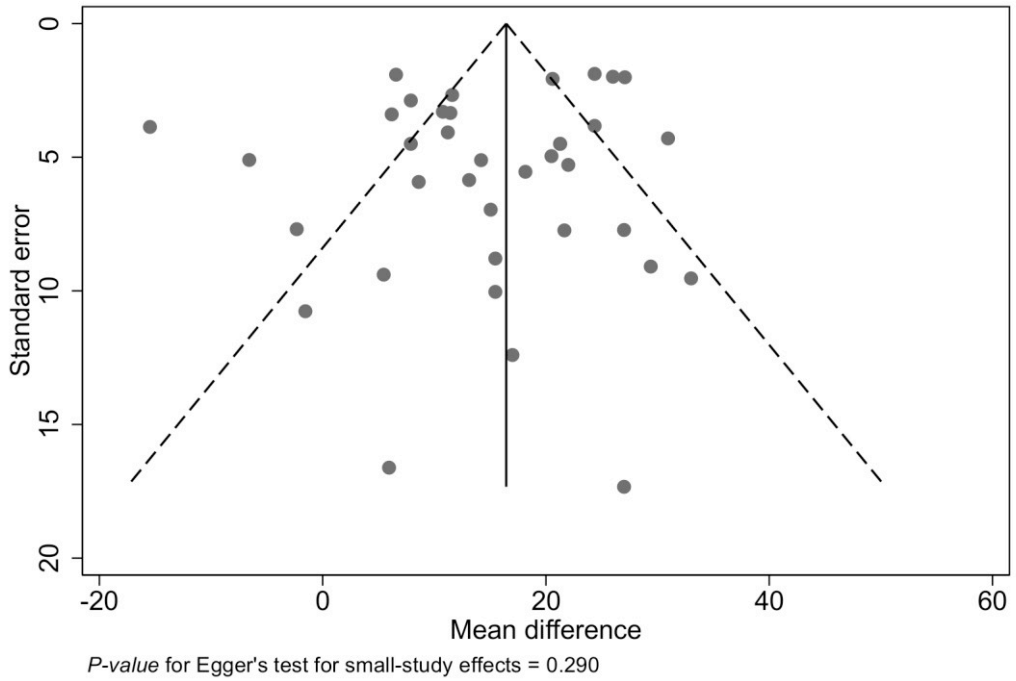
Thirty-seven studies compared mean serum levels of LDL cholesterol between antiretroviral-exposed and antiretroviral-naïve patients, 24 of which reported significantly higher mean serum LDL cholesterol levels in antiretroviral-exposed patients, compared with their treatment-naïve counterparts (Figure 8.3). Overall, the mean serum level of LDL cholesterol among antiretroviral-exposed patients was significantly higher than that among antiretroviral-naïve patients (Pooled MD 14.64 mg/dL, 95% CI 10.83 to 18.45). Heterogeneity across the 37 studies was statistically significant ($P < 0.001$ for Chi^2 test for heterogeneity), and accounted for 86.3% of the variability in the effect estimates between the studies. Funnel plot asymmetry was absent ($P = 0.290$ for Egger's regression test) (Figure 8.4). Sensitivity analysis revealed no instance in which the sequential exclusion of studies altered the interpretation of the pooled association (Table 8.1).

Figure 8.3: Meta-analysis of the association between antiretroviral therapy and low density lipoprotein cholesterol level



ART, antiretroviral therapy; CI, confidence interval; LDLc, low density lipoprotein cholesterol. The black boxes and horizontal lines represent the mean difference in LDLc with 95% CI in each study. The dashed vertical line through the diamond represents the pooled mean difference, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled mean difference. The solid vertical line through zero represents no effect.

Figure 8.4: Analysis of publication bias regarding association between antiretroviral therapy and low-density lipoprotein cholesterol

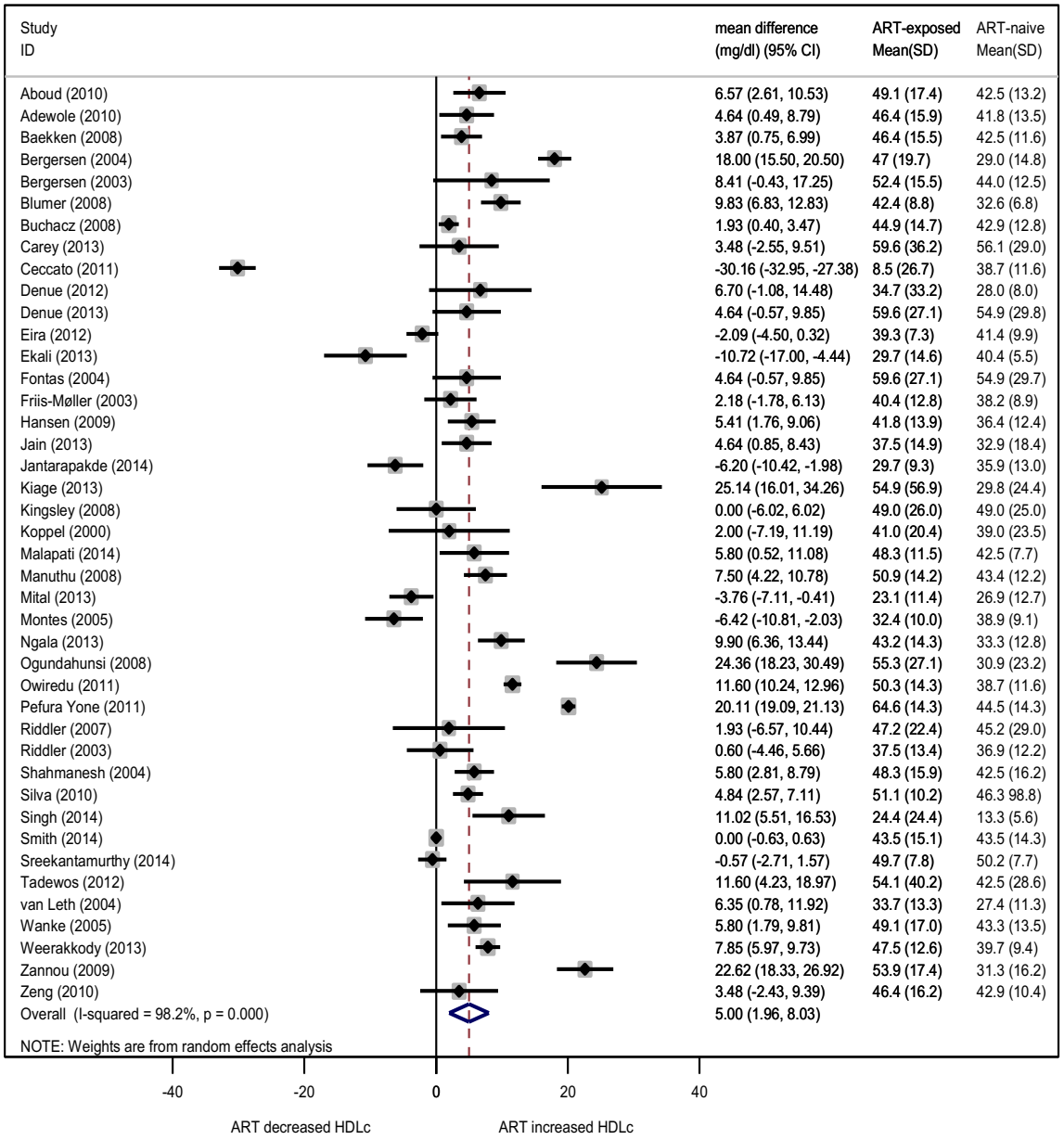


Funnel plot showing mean difference in low density lipoprotein cholesterol for each study plotted against the standard errors. The circles correspond to each study included in the meta-analysis. The solid vertical line represents the pooled effect of antiretroviral therapy on low density lipoprotein cholesterol. The two dashed lines represent the 95% confidence intervals around the pooled effect. The smaller studies represented by circles towards the bottom of the graph are not biased towards a larger effect of antiretroviral therapy on low density lipoprotein cholesterol.

8.1.3 Increase in serum levels of high density lipoprotein cholesterol

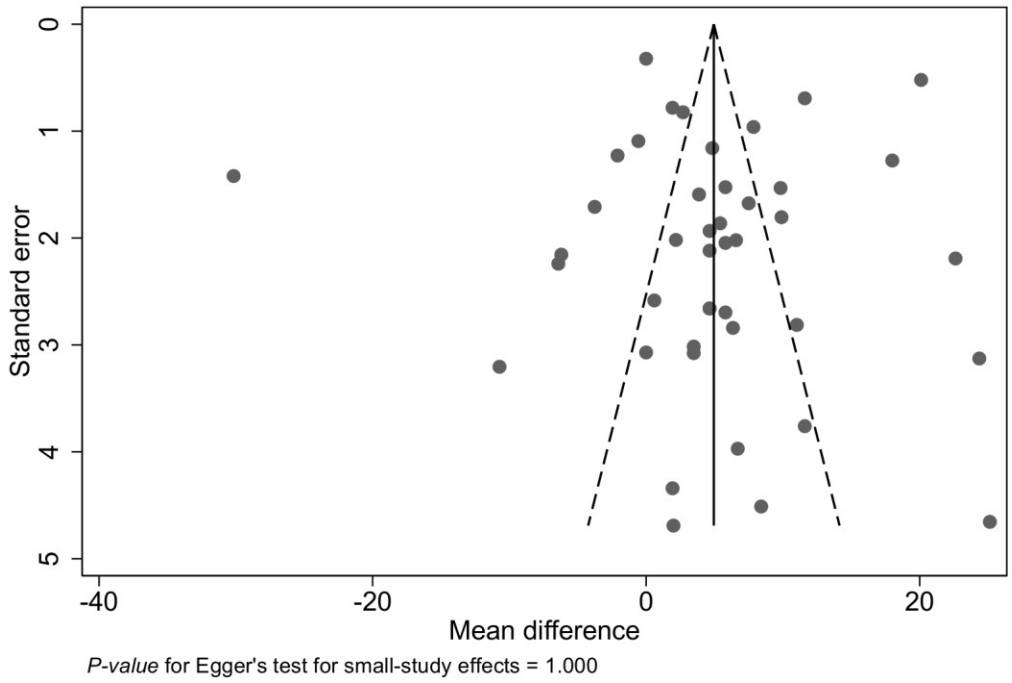
Forty-three studies compared mean serum levels of HDL cholesterol between antiretroviral-exposed and antiretroviral-naïve patients. Overall, patients exposed to antiretroviral therapy had significantly higher mean serum HDL cholesterol concentrations compared with patients naïve to antiretroviral therapy (Pooled MD 5.00 mg/dL, 95% CI 1.96 to 8.03) (Figure 8.5). Heterogeneity was statistically significant across the included studies ($P < 0.001$ for Chi^2 test for heterogeneity), and accounted for 98.2% of between-study variability in the effect estimates. Funnel plot was symmetrical ($P = 1.0$ for Egger's regression test for funnel plot asymmetry) (Figure 8.6). Sensitivity analysis revealed no instance in which the sequential exclusion of studies altered the interpretation of the pooled association (Table 8.1).

Figure 8.5: Meta-analysis of the association between antiretroviral therapy and high density lipoprotein cholesterol level



ART, antiretroviral therapy; CI, confidence interval; HDLc, low density lipoprotein cholesterol. The black boxes and horizontal lines represent the mean difference in HDLc with 95% CI in each study. The dashed vertical line through the diamond represents the pooled mean difference, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled mean difference. The solid vertical line through zero represents no effect.

Figure 8.6: Analysis of publication bias regarding association between antiretroviral therapy and mean high-density lipoprotein cholesterol

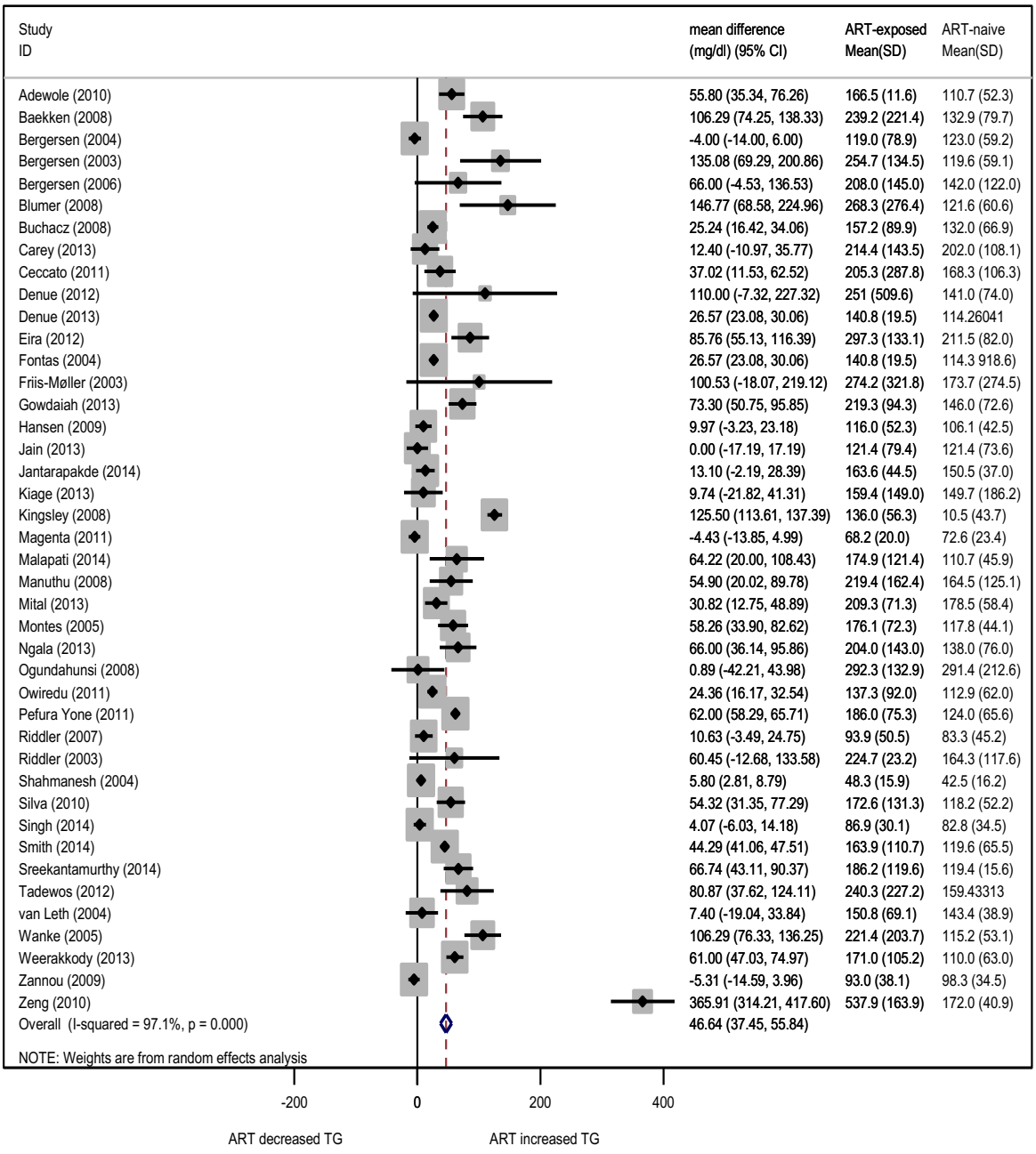


Funnel plot showing mean difference in high density lipoprotein cholesterol for each study plotted against the standard errors. The circles correspond to each study included in the meta-analysis. The solid vertical line represents the pooled effect of antiretroviral therapy on high density lipoprotein cholesterol. The two interrupted diagonal lines represent the 95% confidence intervals around the pooled effect. The smaller studies represented by circles towards the bottom of the graph are not biased towards a larger effect of antiretroviral therapy on high density lipoprotein cholesterol.

8.1.4 Increase in serum levels of triglycerides

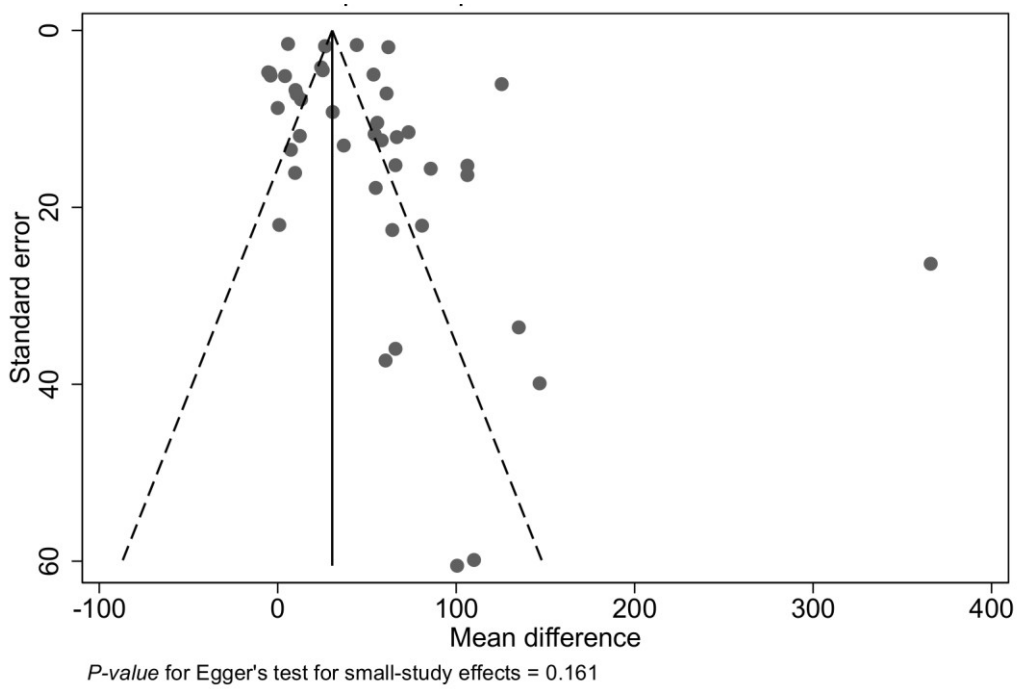
Twenty-seven of 43 studies reported significantly higher mean concentrations of serum triglycerides in patients on antiretroviral therapy, compared to patients who are antiretroviral-naïve (Figure 8.7). Thirteen of the remaining 16 studies also reported higher mean concentrations of serum triglycerides in antiretroviral-exposed patients, even though the differences were not statistically significant. Overall, mean serum concentrations of triglycerides were significantly higher in antiretroviral-exposed patients compared to antiretroviral-naïve patients (Pooled MD 46.64, 95% CI 37.45 to 58.84). Heterogeneity across all 43 studies was statistically significant ($P < 0.001$ for Chi^2 test for heterogeneity) and I^2 statistic was 97.1%. Funnel plot was symmetrical ($P = 0.161$ for Egger's regression test for funnel plot asymmetry), indicating absence of publication bias (Figure 8.8). Sensitivity analysis revealed no instance in which the sequential exclusion of studies altered the interpretation of the pooled association (Table 8.1).

Figure 8.7: Meta-analysis of the association between antiretroviral therapy and serum triglyceride levels



ART, antiretroviral therapy; CI, confidence interval. The black boxes and horizontal lines represent the mean difference in triglyceride levels with 95% CI in each study. The dashed vertical line through the diamond represents the pooled mean difference, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled mean difference. The solid vertical line through zero represents no effect.

Figure 8.8: Analysis of publication of bias in the association between antiretroviral therapy and serum triglyceride levels

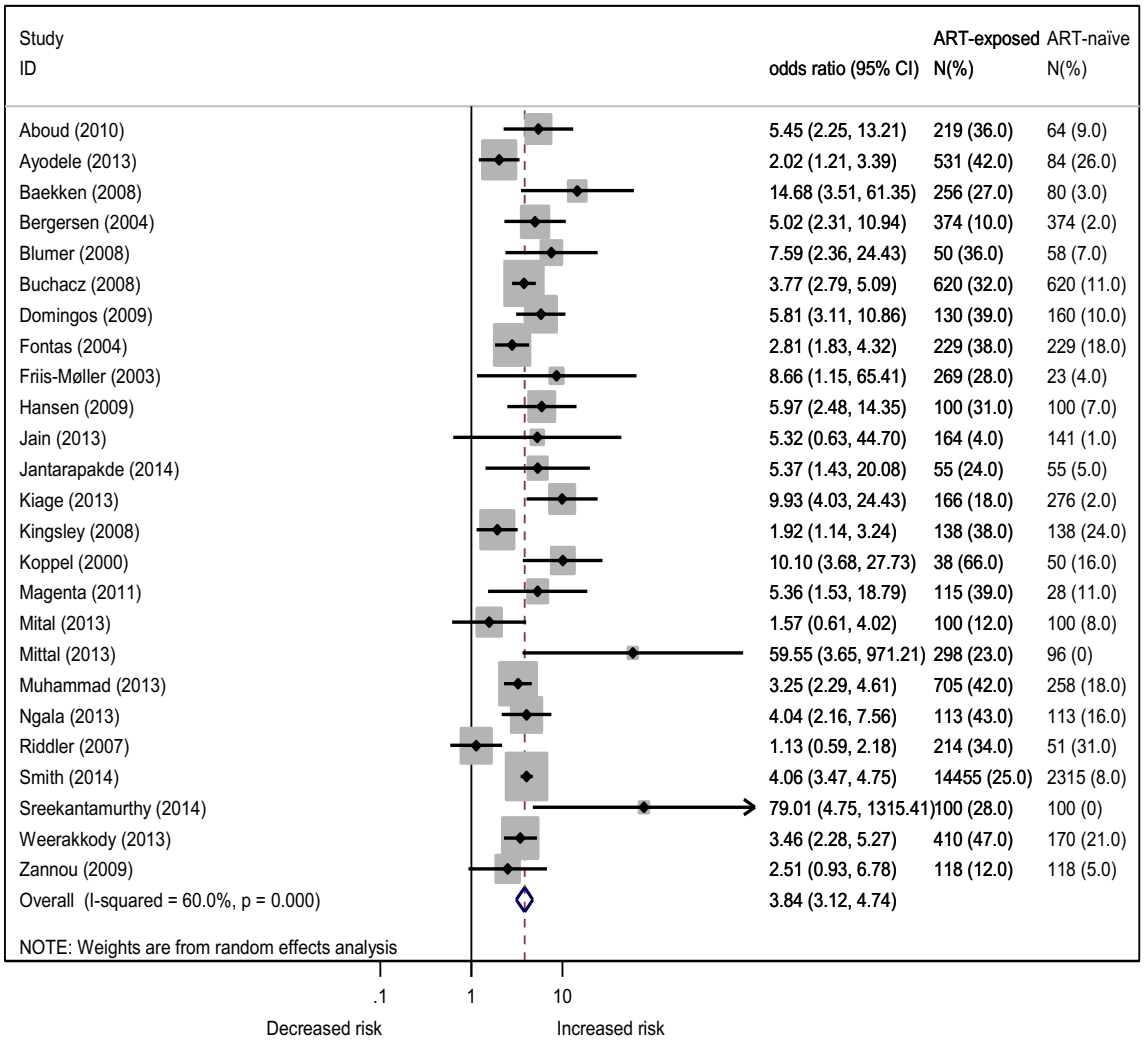


Funnel plot showing mean difference in triglyceride levels for each study plotted against the standard errors. The circles correspond to each study included in the meta-analysis. The solid vertical line represents the pooled effect of antiretroviral therapy on triglycerides. The two dashed lines represent the 95% confidence intervals around the pooled effect.

8.1.5 Increase in the risk of hypercholesterolaemia

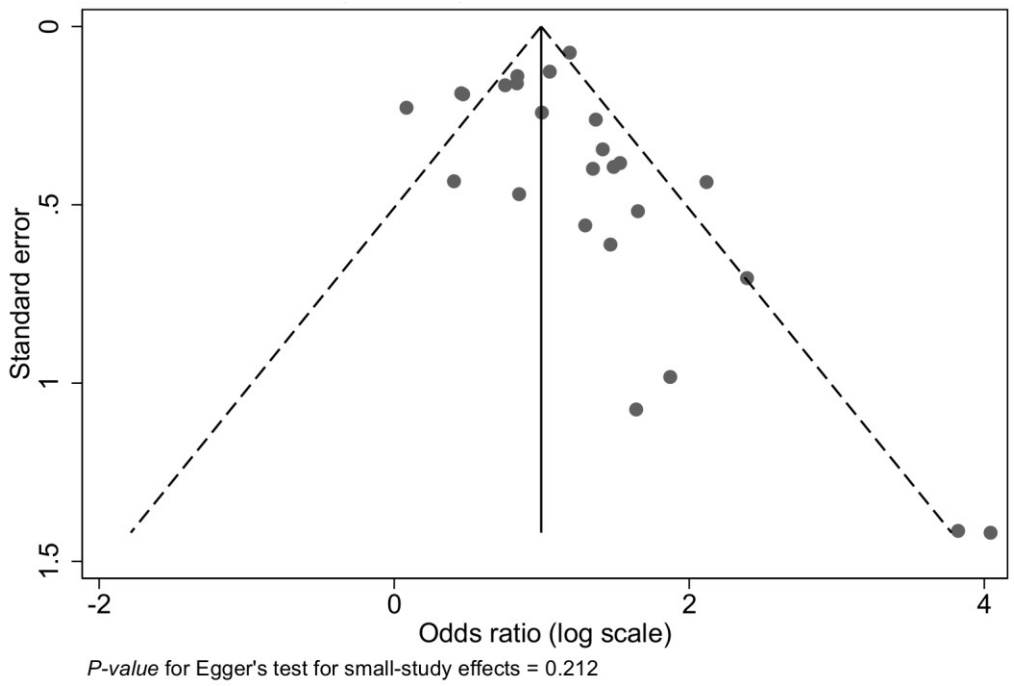
Twenty-five studies compared prevalence estimates of hypercholesterolaemia between patients naïve and exposed to antiretroviral therapy, 21 of which reported statistically significantly higher estimates among those exposed to treatment (Figure 8.9). The other four studies also reported higher prevalence estimates of hypercholesterolaemia in antiretroviral-exposed patients than their naïve counterparts, although these differences were not statistically significant (Jain *et al*., 2013; Mital *et al*., 2013; Riddler *et al*., 2007; Zannou *et al*., 2009). Overall, hypercholesterolaemia was found in 5,433 (27.2%) of 19,967 HIV-infected patients exposed to antiretroviral therapy hypercholesterolaemia compared to 539 (9.3%) of 5,801 HIV-infected patients naïve to antiretroviral therapy, so that the odds of hypercholesterolaemia were almost four times higher among antiretroviral-treated patients than their naïve counterparts (Pooled OR 3.84, 95% CI 3.12 to 4.74). Heterogeneity across all 25 studies was statistically significant ($P < 0.001$), and explained 60% of the variability in the odds ratios between individual studies. Funnel plot was symmetrical ($P = 0.212$ for Egger's regression test for funnel plot asymmetry), indicating the absence of publication bias (Figure 8.10). Sensitivity analysis revealed no instance in which the sequential exclusion of studies altered the interpretation of the pooled association (Table 8.1).

Figure 8.9: Meta-analysis of the association between antiretroviral therapy and hypercholesterolaemia



ART, antiretroviral therapy; CI, confidence interval. The black boxes and horizontal lines represent the odds ratios of hypercholesterolaemia with 95% CI in each study. The interrupted vertical line through the diamond represents the pooled odds ratio, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled odds ratio. The solid vertical line through zero represents no effect.

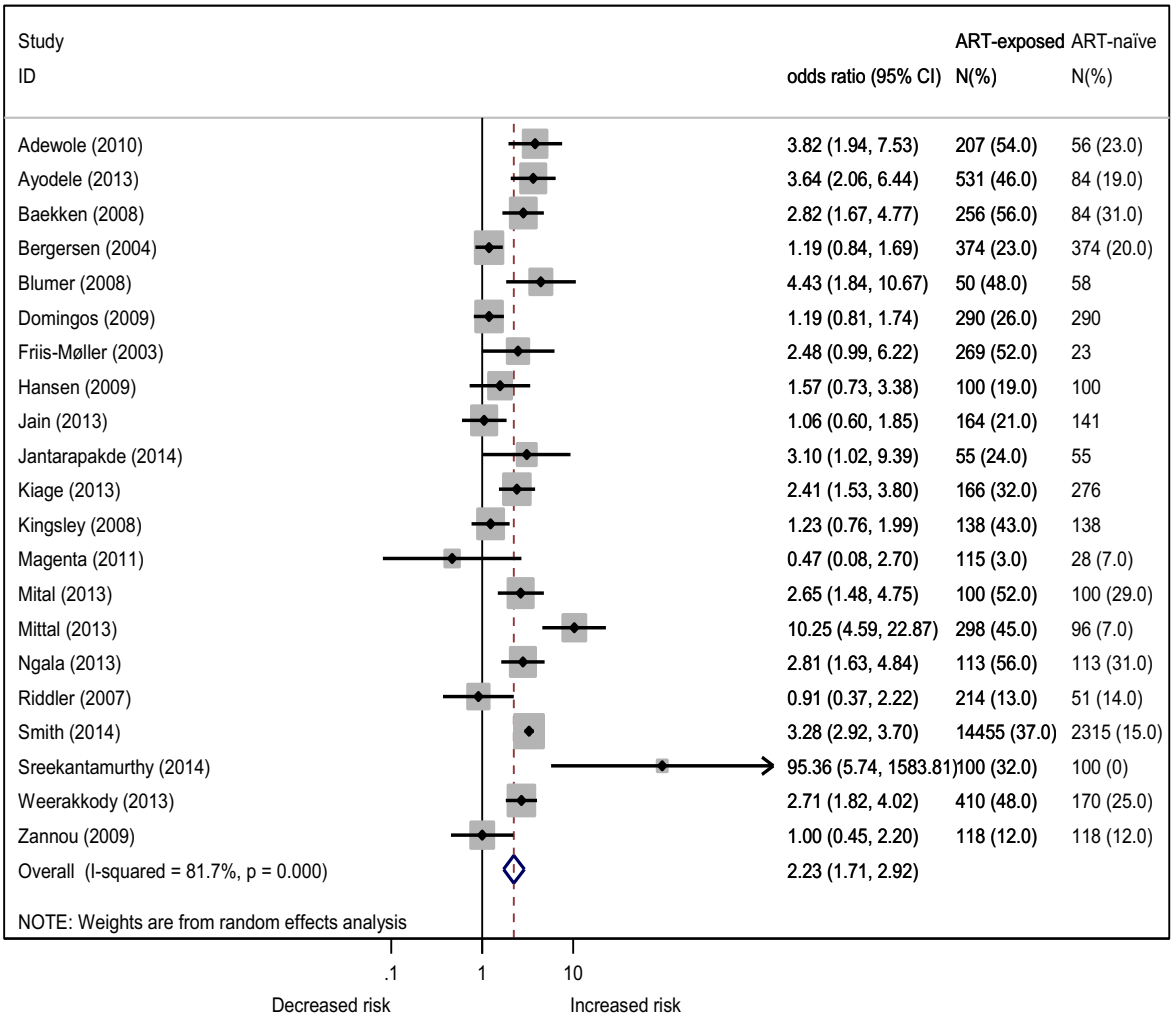
Figure 8.10: Analysis of publication bias in the association between antiretroviral therapy and the risk of hypercholesterolemia



8.1.6 Increase in the risk of hypertriglyceridaemia

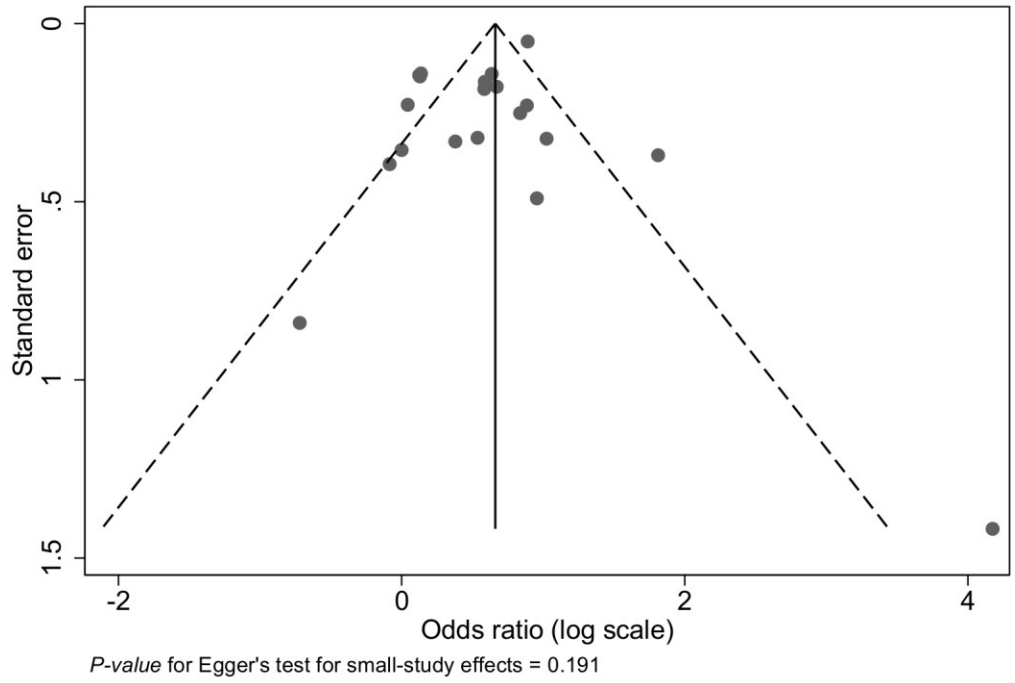
Prevalence estimates of hypertriglyceridaemia were compared between antiretroviral-exposed and antiretroviral-naïve HIV-infected patients in 21 studies, 12 of which reported significantly higher estimates in the antiretroviral-exposed group (Figure 8.11). Seven of the remaining nine studies also reported higher prevalence estimates of hypertriglyceridaemia in antiretroviral-exposed patients compared to their naïve counterparts, however these differences were not statistically significant. Although prevalence estimates of hypertriglyceridaemia were lower among antiretroviral-exposed patients than their naïve counterparts in two studies, these differences were not statistically significant (Ayodele *et al*., 2013; Ekali *et al*., 2013). Overall, hypertriglyceridaemia was reported in 6,879 of 18,523 (37.1%) antiretroviral-exposed patients, as opposed to 845 of 4,770 (17.7%) antiretroviral-naïve patients, such that the odds of hypertriglyceridaemia were more than two times higher in antiretroviral-exposed patients compared with their naïve counterparts (Pooled OR 2.23, 95% CI 1.71 to 2.92). Heterogeneity across the 21 studies was statistically significant ($P < 0.001$ for Chi^2 test for heterogeneity) and accounted for a considerable amount of between-study variability in the effect estimates ($I^2 = 81.7\%$). Funnel plot was symmetrical ($P = 0.191$ for Egger's regression test for funnel plot asymmetry) (Figure 8.12). Sensitivity analysis revealed no instance in which the sequential exclusion of studies altered the interpretation of the pooled association (Table 8.1).

Figure 8.11: Meta-analysis of the association between antiretroviral therapy and hypertriglyceridaemia



ART, antiretroviral therapy; CI, confidence interval. The black boxes and horizontal lines represent the odds ratios of hypertriglyceridaemia with 95% CI in each study. The dashed vertical line through the diamond represents the pooled odds ratio, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled odds ratio. The solid vertical line through zero represents no effect.

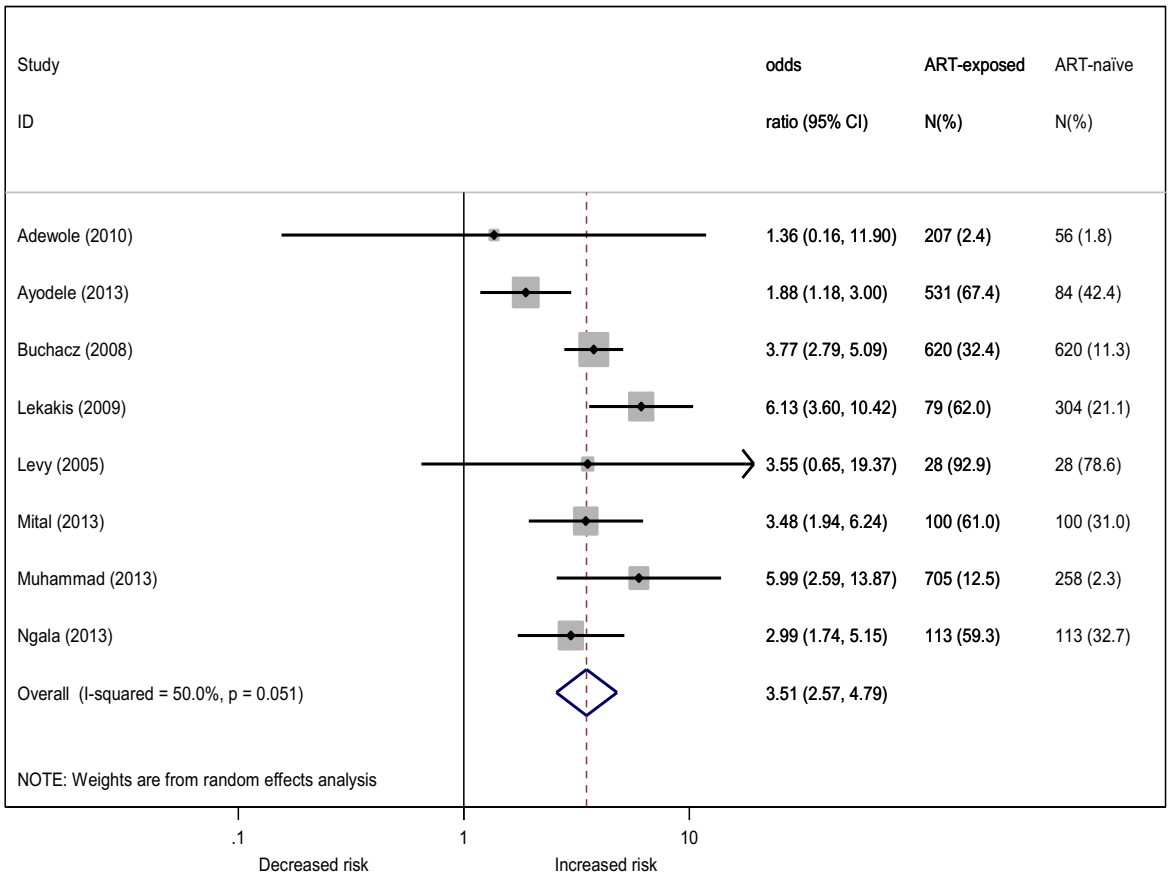
Figure 8.12: Analysis of publication bias regarding the association between antiretroviral therapy and the risk of hypertriglyceridaemia



8.1.7 Increase in the risk of combined dyslipidaemia

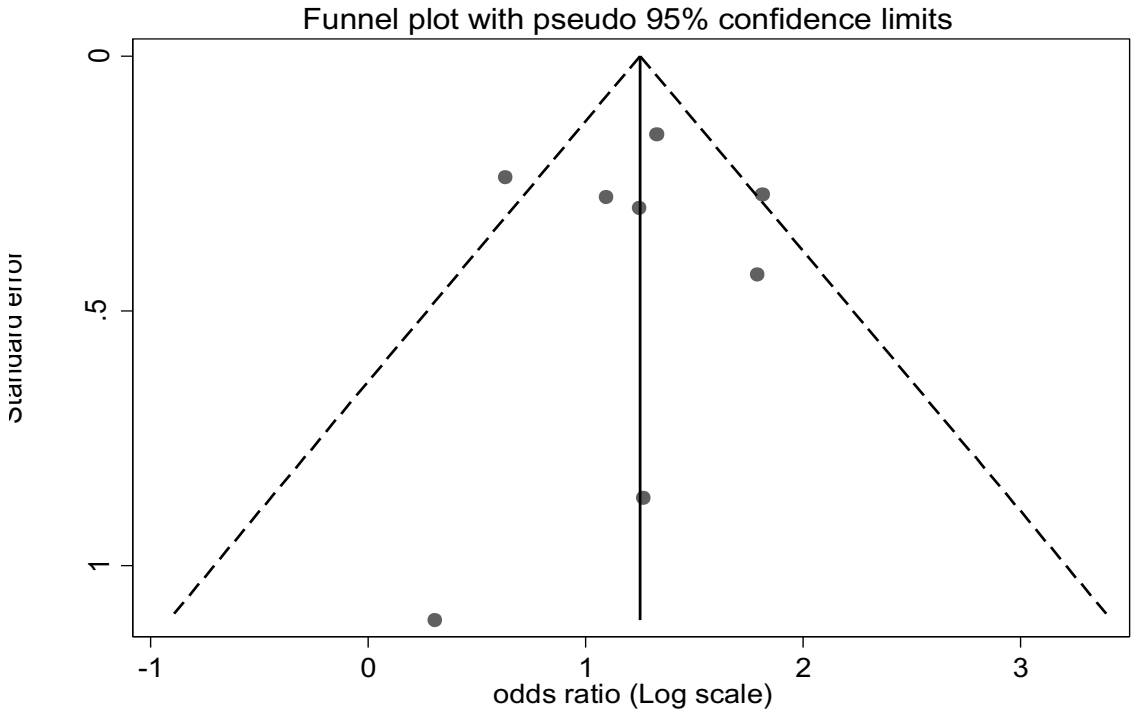
Eight studies compared prevalence estimates of combined dyslipidaemia between antiretroviral-exposed and antiretroviral-naïve patients (Adewole *et al*., 2010; Ayodele *et al*., 2013; Buchacz *et al*., 2008; Lekkaki *et al*., 2009; Levy *et al*., 2005; Mital *et al*., 2013; Muhammad *et al*., 2013a; Ngala *et al*., 2013). Prevalence estimates of combined dyslipidaemia were higher among patients on antiretroviral therapy compared with antiretroviral-naïve patients in all eight studies, six of which reported a statistically significant difference (Ayodele *et al*., 2013; Buchacz *et al*., 2008; Lekkaki *et al*., 2009; Mital *et al*., 2013; Muhammad *et al*., 2013a; Ngala *et al*., 2013) (Figure 8.13). Overall, combined dyslipidaemia was reported in 855 of 2,383 (35.9%) antiretroviral-exposed HIV-infected patients, as opposed to 275 of 1,563 (17.6%) antiretroviral-naïve patients, so that the odds of having dyslipidaemia were more than three times higher in antiretroviral-treated patients than in antiretroviral-naïve patients (Pooled OR 3.51, 95% CI 2.57 to 4.79). Heterogeneity across the eight studies was not statistically significant ($P = 0.051$), but accounted for 50% of between-study variability in the effect estimates. No evidence of publication bias was found ($P = 0.927$ for Egger's regression test for funnel plot asymmetry) (Figure 8.14). Sensitivity analysis revealed no instance in which the sequential exclusion of studies altered the interpretation of the pooled association (Table 8.1).

Figure 8.13: Meta-analysis of the association between antiretroviral therapy and combined dyslipidaemia



ART, antiretroviral therapy; CI, confidence interval. The grey boxes and horizontal lines represent the odds ratios of dyslipidaemia with 95% CI in each study. The dashed vertical line through the diamond represents the pooled odds ratio, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled odds ratio. The solid vertical line through zero represents no effect.

Figure 8.14: Analysis of publication bias in the association between antiretroviral therapy and combined dyslipidaemia



P = 0.927 for Egger's test for small-study effects.

Table 8.1: Sensitivity analysis of the pooled associations of antiretroviral therapy with increased serum lipoprotein levels and dyslipidaemias

Cardio-metabolic outcomes	Pooled estimate prior to serial exclusion of individual studies Pooled ES (95% CI)	Range of pooled estimate following serial exclusion of individual studies	Instance in which exclusion of a study changed interpretation of the pooled association Pooled ES (95% CI)
Total cholesterol	29.41 (26.47 to 32.35)	28.76 to 30.25	None
LDL cholesterol	14.85 (11.23 to 18.47)	14.33 to 15.89	None
HDL cholesterol	4.94 (2.03 to 7.85)	4.49 to 5.80	None
Triglycerides	46.81 (37.38 to 55.84)	41.52 to 48.22	None
Hypercholesterolaemia	3.84 (3.12 to 4.74)	3.70 to 4.00	None
Hypertriglyceridaemia	2.23 (1.71 to 2.92)	2.08 to 2.33	None
Combined dyslipidaemia	3.51 (2.57 to 4.79)	3.51 to 3.96	None

CI, confidence interval; Pooled ES, Pooled estimate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

8.2 FACTORS THAT MAY INFLUENCE THE ASSOCIATIONS OF ANTIRETROVIRAL THERAPY WITH INCREASED SERUM LIPID LEVELS

Table 8.2 presents the results of subgroup and meta-regression analyses of the association between antiretroviral therapy and total cholesterol levels. There were significant differences in the impact of antiretroviral therapy on total cholesterol levels by country income group, age group, smoking status, CD4 cell count, antiretroviral regimen, and year of publication. The, studies conducted in high-income countries reported a stronger association between antiretroviral therapy and increased total cholesterol levels (Pooled MD 36.53 mg/dL, 95% CI 32.62 to 40.41), compared to studies conducted in low- and middle-income countries (Pooled 25.37 mg/dL, 95% CI 21.57 to 28.95) ($P = 0.002$ for interaction). The impact of antiretroviral therapy on total cholesterol was significantly higher among studies where the average age of the participants was 40 years or more (Pooled MD 35.43 mg/dL, 95% CI 29.58 to 41.22), compared to studies where the average age was below 40 years old (Pooled MD 27.41 mg/dL, 95% CI 23.97 to 30.83) ($P = 0.027$ for interaction). Studies comprising larger proportions of smokers than non-smokers reported significantly higher increases in serum total cholesterol levels associated with antiretroviral therapy (Pooled MD 39.16 mg/dL, 95% CI 23.17 to 55.14), compared to studies comprising smaller proportions of smokers than non-smokers (Pooled MD 24.22 mg/dL, 95% CI 18.42 to 30.03) ($P = 0.048$ for interaction). Studies where mean CD4 cell count was 350 cells/mm^3 or greater reported stronger association between antiretroviral therapy and increased serum total cholesterol level (Pooled MD 33.81 mg/dL, 95% CI 29.50 to 38.13), compared to studies with mean CD4 count less than 350 cells/mm^3 (Pooled MD 25.78 mg/dL, 95% CI 22.37 to 29.18) ($P = 0.043$ for interaction). The pooled association between antiretroviral therapy and increased total cholesterol level was also significantly stronger with the use of protease inhibitors (Pooled MD 32.29 mg/dL, 95%

CI 28.43 to 36.16), as opposed to the use of non-protease inhibitor-based antiretroviral regimens (Pooled MD 24.25 mg/dL, 95% CI 19.41 to 29.09) (P = 0.037 for interaction). Studies published prior to 2010 reported a significantly larger impact of antiretroviral therapy on total cholesterol level (Pooled MD 34.7 mg/dL, 95% CI 31.0 to 38.3), compared to studies published from 2010 onwards (Pooled MD 24.9 mg/dL, 95% CI 20.5 to 29.2) (P = 0.005 for interaction). Among these study level factors, differences by country income group accounted for most of the variability between studies in the effects of antiretroviral therapy on total cholesterol levels (R^2 = 24%). Importantly, given that the risk of selection bias was high or unclear in 74% of all the studies, the impact of selection bias on the pooled association was also examined using subgroup and meta-regression analyses, which revealed no statistically significant difference between studies assessed to have high or unclear risk of bias (Pooled MD 27.31 mg/dL, 95% CI 25.32 to 29.30) and studies assessed to have a low risk of selection bias (Pooled MD 33.42 mg/dL, 95% CI 32.20 to 34.51) (P = 0.585 for interaction). There was also no significant variation in the pooled association between antiretroviral therapy and total cholesterol levels by other study-level subgroups, including geographical region, sex, duration of HIV infection, duration of antiretroviral therapy and study design.

Table 8.2: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and serum total cholesterol level

Subgroup	N	Pooled MD (95% CI)	Meta-regression	
			P value	R^2 (%)
Region				
Sub-Saharan Africa	14	23.06 (17.44 to 28.68)		
Europe	15	36.77 (32.74 to 48.80)		
South-East Asia	9	29.68 (22.57 to 36.79)		
The Americas	5	26.26 (20.15 to 32.37)		
Western Pacific	1	4.64 (-8.67 to 17.96)	0.834	0
Income group				
High income	17	36.53 (32.62 to 40.41)		
Low/middle	28	25.37 (21.57 to 28.95)	0.002	24
Females				
< 50%	28	31.66 (28.26 to 35.07)		
≥ 50%	13	24.50 (17.85 to 31.16)	0.118	6.7
Age group				
< 40 years	26	27.41 (23.97 to 30.83)		
≥ 40 years	17	35.43 (29.58 to 41.22)	0.027	11.5
Smokers				
< 50%	17	24.22 (18.42 to 30.03)		
≥ 50%	6	39.16 (23.17 to 55.14)	0.048	17.3
CD4 count				
< 350 cells/mm^3	18	25.78 (22.37 to 29.18)		
≥ 350 cells/mm^3	17	33.81 (29.50 to 38.13)	0.043	14.8
HIV duration				
< 60 months	5	34.77 (23.87 to 45.67)		
≥ 60 months	9	33.57 (24.78 to 42.37)	0.871	0
ART regimen				
PI-based	19	32.29 (28.43 to 36.16)		
Non-PI-based	19	24.25 (19.41 to 29.09)	0.037	9.7
ART duration				
< 18 months	11	23.07 (13.79 to 32.35)		
≥ 18 months	31	30.12 (26.99 to 33.26)	0.114	2.7
Study design				
Cross-sectional	26	30.82 (26.15 to 35.41)		
Cohort	19	27.73 (23.62 to 31.93)	0.484	0
Selection bias				
Low risk	17	33.42 (32.20 to 34.51)		
High/unclear risk	27	27.31 (25.32 to 29.30)	0.585	0
Publication year				
2000–2009	20	34.74 (30.96 to 38.31)		
2010–2014	25	24.86 (20.49 to 29.24)	0.005	19.1

ART, antiretroviral therapy; MD, mean difference; N, number of studies; PI, protease inhibitor; R^2, explained variability

Table 8.3 presents the results of subgroup and meta-regression analyses of the association between antiretroviral therapy and LDL cholesterol level. The impact of antiretroviral therapy on LDL cholesterol level was significantly higher among studies with 200 participants or more (Pooled MD 17.62 mg/dL, 95% CI 13.24 to 21.99), compared to studies with less than 200 participants (Pooled MD 10.29 mg/dL 4.27 to 16.31) ($P = 0.046$ for interaction). Studies published prior to 2010 were more likely to report a stronger association between antiretroviral therapy and increased LDL cholesterol levels (Pooled MD 20.38 mg/dL, 95% CI 16.02 to 24.78), compared to studies published from 2010 onwards (Pooled MD 11.97 mg/dL, 95% CI 7.61 to 16.42) ($P = 0.030$ for interaction). Difference in the pooled association by country income group was of borderline statistical significance: studies conducted in high income countries were more likely to report stronger associations between antiretroviral therapy and increased LDL cholesterol Pooled (MD 21.42 mg/dL, 95% CI 16.21 to 26.52), compared to studies conducted in low- and middle-income countries (Pooled MD 12.88 mg/dL, 95% CI 8.67 to 17.09) ($P = 0.056$ for interaction). Of note, there was no statistically significant difference in the pooled association between studies assessed to have high or unclear risk of selection bias (Pooled MD 15.83 mg/dL, 95% CI 13.91 to 17.62) and studies assessed to have low risk of selection bias (Pooled MD 16.32 mg/dL, 95% CI 14.53 to 18.01) ($P = 0.881$ for interaction).

Table 8.3: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and serum low-density lipoprotein cholesterol level

Subgroup	N	Pooled MD (95% CI)	Meta-regression	
			P value	R^2 (%)
Region				
Sub-Saharan Africa	13	15.36 (7.37 to 23.36)		
Europe	8	21.77 (16.13 to 21.42)		
South-East Asia	8	12.40 (9.03 to 15.76)		
The Americas	6	7.53 (4.35 to 10.72)		
Western Pacific	1	-6.57 (-16.58 to 3.43)	0.083	8.3
Income group				
High	10	21.42 (16.21 to 26.52)		
Low/middle	26	12.88 (8.67 to 17.09)	0.056	11.2
Females				
< 50%	20	14.85 (10.27 to 19.42)		
> 50%	12	13.24 (4.93 to 21.55)	0.670	0
Age group				
< 40 years	23	14.23 (9.89 to18.63)		
≥ 40 years	12	19.69 (16.52 to 23.98)	0.208	5.1
Smokers				
< 50%	16	11.46 (5.02 to 17.89)		
≥ 50%	3	1.68 (-13.60 to 16.95)	0.314	4.2
CD4 count				
< 350 cells/mm^3	17	16.63 (12.32 to 20.95)		
≥ 350 cells/mm^3	11	16.64 (9.05 to 24.24)	0.988	0
HIV duration				
< 60 months	5	12.16 (7.77 to 16.55)		
≥ 60 months	6	19.95 (3.43 to 24.47)	0.681	0
ART regimen				
PI-based	14	12.56 (6.83 to 18.30)		
Non-PI-based	18	15.75 (10.05 to 21.44)	0.447	0
ART duration				
< 18 months	19	10.03 (0.93 to 19.14)		
≥ 18 months	25	16.09 (11.86 to 20.31)	0.159	3.2
Study design				
Cross-sectional	20	15.24 (9.79 to 20.48)		
Cohort	17	14.51 (9.30 to 19.69)	0.859	0
Selection bias				
Low risk	14	15.83 (13.91 to 17.62)		
High/unclear risk	22	16.32 (14.53 to 18.01)	0.881	0
Year of publication				
2000–2009	13	20.38 (16.02 to 24.78)		
2010–2015	24	11.97 (7.61 to 16.42)	0.030	15

ART, antiretroviral therapy; MD, mean difference; N, number of studies; PI, protease inhibitor; R^2, explained variability

Table 8.4 presents the results of subgroup and meta-regression analyses of the association between antiretroviral therapy and HDL cholesterol level. Antiretroviral therapy was associated with a statistically significant increase in HDL cholesterol level across subgroups of different study-level characteristics including age group, smoking status, HIV infection duration and antiretroviral treatment duration. In addition, no statistically significant difference in the pooled estimate was observed between subgroups of these characteristics.

Antiretroviral therapy was also associated with increased HDL cholesterol levels across subgroups of other study-level characteristics, however some of these associations were not statistically significant. For instance, antiretroviral therapy was significantly associated with increased HDL cholesterol levels among studies with more women than men (Pooled MD 8.65 mg/dL, 95% CI 5.63 to 11.63), but not among studies with fewer women than men (Pooled MD 3.92 mg/dL, 95% CI -0.22 to 8.06). Nonetheless, the difference between both subgroup estimates was not statistically significant (P = 0.157 for interaction). Similarly, antiretroviral therapy was significantly associated with increased HDL cholesterol levels among HIV-infected patients with CD4 cell counts greater than 350 cells/mm^3 (Pooled MD 5.89 mg/dL, 95% CI 1.02 to 10.76), but not among HIV-infected patients with CD4 cell counts less than 350 cells/mm^3 (Pooled MD 4.69 mg/dL, 95% CI -2.80 to 12.18) (P = 0.687 for interaction). Antiretroviral therapy was also significantly associated with increased HDL cholesterol levels among HIV-infected patients on non-protease inhibitor-based antiretroviral regimens (Pooled MD 7.25 mg/dL, 95% CI 3.39 to 11.12), but not among those on protease inhibitors (Pooled MD 1.87, 95% CI -3.85 to 7.59) (P = 0.115 for interaction).

Table 8.4: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and high-density lipoprotein cholesterol level

Subgroup	N	Pooled MD (95% CI)	Meta-regression	
			P value	R^2 (%)
Region				
Sub-Saharan Africa	13	9.51 (4.38 to 14.65)		
Europe	13	4.60 (-2.30 to 11.49)		
South-East Asia	10	1.20 (-2.41 to 4.81)		
The Americas	5	3.10 (0.47 to 5.73)		
Western Pacific	1	3.48 (-2.43 to 9.39)	0.066	5.9
Income group				
High	15	4.23 (-2.20 to 10.61)		
Low/middle	28	5.29 (2.83 to 7.84)	0.692	11.2
Females				
< 50%	26	3.92 (-0.22 to 8.06)		
≥ 50%	12	8.65 (5.63 to 11.63)	0.157	2.8
Age group				
< 40 years	25	4.89 (0.71 to 9.20)		
≥ 40 years	15	3.91 (2.20 to 5.56)	0.832	5.1
Smokers				
< 50%	18	7.74 (3.45 to 12.03)		
≥ 50%	5	5.50 (3.41 to 7.59)	0.506	0
CD4 count				
< 350 cells/mm^3	16	4.69 (-2.80 to 12.18)		
≥ 350 cells/mm^3	16	5.89 (1.02 to 10.76)	0.687	0
HIV duration				
< 60 months	6	4.30 (0.45 to 8.15)		
≥ 60 months	9	4.88 (2.29 to 7.47)	0.807	0
ART regimen				
PI-based	17	1.87 (-3.85 to 7.59)		
Non-PI-based	19	7.25 (3.39 to 11.12)	0.115	4.5
ART duration				
< 18 months	10	7.58 (1.94 to 13.22)		
≥ 18 months	30	3.82 (0.03 to 7.62)	0.278	0.4
Study design				
Cross-sectional	25	5.13 (1.52 to 8.67)		
Cohort	18	4.82 (0.41 to 9.16)	0.919	0
Selection bias				
Low risk	16	5.75 (5.26 to 6.23)		
High/unclear risk	26	3.41 (2.73 to 4.12)	0.109	4.6
Year of publication				
2010–2014	25	5.10 (2.67 to 7.51)		
2000–2009	18	4.49 (-1.22 to 10.18)	0.790	0

ART, antiretroviral therapy; MD, mean difference; N, number of studies; PI, protease inhibitor; R^2, explained variability

Table 8.5 presents the results of subgroup and meta-regression analyses of the association between antiretroviral therapy and triglyceride levels. Significant regional differences in the impact of antiretroviral therapy on serum triglyceride levels were observed, accounting for 28% of between-study variability. With the one study from the Western Pacific region reporting a massive difference in mean serum triglyceride levels between antiretroviral-exposed and antiretroviral-naïve patients (537.89 mg/dL *versus* 163.87 mg/dL) (Zeng *et al*., 2010), it may appear that differences across regions were driven by the effect estimate from the Western Pacific region: the mean difference in serum triglyceride levels between HIV-infected patients naïve and exposed to antiretroviral therapy obtained in the Western Pacific region was no less than six times greater than the pooled mean differences obtained from the other regions ($P < 0.001$ for interaction). However, regional differences persisted even after excluding Zeng *et al*. (2010) from the meta-analysis ($P = 0.029$ for interaction; $R^2 = 9.7\%$). Studies comprising larger proportions of smokers than non-smokers reported significantly higher increases in serum total cholesterol levels associated with antiretroviral therapy (Pooled MD 141.54 mg/dL, 95% CI 39.48 to 243.60), compared to studies comprising smaller proportions of smokers than non-smokers (Pooled MD 34.08 mg/dL, 95% CI 19.53 to 48.63) ($P = 0.005$ for interaction). Studies with mean CD4 cell counts of 350 cells/mm^3 were more likely to report stronger associations between antiretroviral therapy and increased serum triglyceride levels (Pooled MD 72.85 mg/dL, 95% CI 54.92 to 90.79), compared to studies with mean CD4 cell counts less than 350 cells/mm^3 (Pooled MD 29.39 mg/dL, 95% CI 16.96 to 41.83) ($P = 0.055$ for interaction).

Table 8.5: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and serum triglyceride level

Subgroup	N	Pooled MD (95% CI)	Meta-regression	
			P value	R^2 (%)
Region				
Sub-Saharan Africa	14	18.97 (1.71 to 36.24)		
Europe	14	61.47 (44.12 to 78.82)		
South-East Asia	8	51.36 (34.35 to 68.40)		
The Americas	4	39.66 (15.43 to 63.89)		
Western Pacific	1	365.91 (314.21 to 417.60)	0.000	28.4
Income group				
Low/middle	28	40.91 (29.45 to 52.42)		
High	15	61.39 (44.44 to 78.50)	0.324	0.5
Females				
< 50%	26	54.64 (41.48 to 67.80)		
≥ 50%	12	33.56 (12.27 to 54.85)	0.265	0.8
Age group				
< 40 years	24	45.23 (31.52 to 58.78)		
≥ 40 years	15	52.77 (40.84 to 64.67)	0.507	0
Smokers				
< 50%	17	34.08 (19.53 to 48.63)		
≥ 50%	5	141.54 (39.48 to 243.60)	0.005	35.0
CD4 count				
< 350 cells/mm^3	18	29.39 (16.96 to 41.83)		
≥ 350 cells/mm^3	15	72.85 (54.92 to 90.79)	0.055	11.4
HIV duration				
< 60 months	7	62.99 (34.20 to 91.78)		
≥ 60 months	8	58.14 (31.08 to 85.20)	0.759	0
ART regimen				
PI-based	17	70.79 (55.36 to 84.21)		
Non-PI-based	20	24.70 (13.48 to 35.91)	0.005	21.0
ART duration				
< 18 months	11	30.16 (17.22 to 43.10)		
≥ 18 months	28	52.96 (40.72 to 65.20)	0.322	0
Study design				
Cross-sectional	25	62.54 (44.67 to 80.31)		
Cohort	18	29.71 (21.03 to 38.42)	0.133	4.1
Selection bias				
Low risk	16	38.1 (36.3 to 39.9)		
High/unclear risk	26	20.7 (18.7 to 22.7)	0.544	0
Year of publication				
2010–2015	25	46.42 (33.89 to 58.78)		
2000–2009	18	49.11 (34.08 to 64.21)	0.902	0

ART, antiretroviral therapy; MD, mean difference; N, number of studies; PI, protease inhibitor; R^2, explained variability

Table 8.6 presents the results of subgroup and meta-regression analyses of the association between antiretroviral therapy and the odds of hypercholesterolaemia. The results showed no statistically significant difference between subgroup estimates of the association of antiretroviral therapy with increased risk of hypercholesterolaemia. The duration of antiretroviral therapy accounted for most of the between-study variability, with the impact of antiretroviral therapy on the risk of hypercholesterolaemia being approximately twice as high among HIV-infected patients who received antiretroviral therapy for 18 months or more (Pooled OR 3.86, 95% CI 3.28 to 4.55), compared to HIV-infected patients on antiretroviral therapy for less than 18 months (Pooled OR 2.02, 95% CI 0.83 to 7.02).

Table 8.6: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and the odds of hypercholesterolaemia

			Meta-regression	
Subgroup	**N**	**Pooled OR (95% CI)**	***P* value**	**R^2 (%)**
Region				
Sub-Saharan Africa	12	3.66 (2.55to 5.27)		
Europe	5	4.43 (3.04 to 6.46)		
South-East Asia	4	4.41 (1.72 to 11.26)		
The Americas	4	3.98 (2.15 to 7.37)	0.419	0
Income group				
High	7	3.61 (2.10 to 6.08)		
Low/middle	18	3.64 (2.82 to 4.82)	0.512	0
Females				
< 50%	11	4.04 (3.09 to 5.26)		
≥ 50%	12	4.00 (2.78 to 5.75)	0.141	5.8
Age group				
< 40 years	17	3.73 (2.84 to 4.83)		
≥ 40 years	5	3.67 (2.03 to 6.82)	0.928	0
Smokers				
< 50%	11	3.72 (2.66 to 5.21)		
≥ 50%	2	3.11 (1.18 to 8.20)	0.635	0
CD4 count				
< 350 cells/mm^3	9	2.93 (1.86 to 4.60)		
≥ 350 cells/mm^3	9	4.27 (3.35 to 5.42)	0.203	4.3
ART regimen				
PI-based	7	4.30 (3.43 to 5.38)		
Non-PI-based	13	3.48 (2.35 to 5.16)	0.713	0
ART duration				
< 18 months	4	2.02 (0.83 to 7.02)		
≥ 18 months	17	3.86 (3.28 to 4.55)	0.094	9.4
Study design				
Cohort	8	3.52 (2.63 to 4.56)		
Cross-sectional	17	3.78 (2.64 to 5.51)	0.643	0
Year of publication				
2000–2009	10	4.80 (3.04 to 7.82)		
2010–2015	15	3.13 (2.32 to 4.23)	0.820	0

ART, antiretroviral therapy; N, number of studies; OR, odds ratio; PI, protease inhibitor; R^2, explained variability. HIV infection duration was reported in less than ten studies and was excluded from the meta-regression analyses.

Table 8.7 presents the results of subgroup and meta-regression analyses of the association between antiretroviral therapy and the odds of hypertriglyceridaemia. With the exception of CD4 cell count, there was no statistically significant difference between subgroup estimates of the association of antiretroviral therapy with increased risk of hypertriglyceridaemia. CD4 cell count accounted for most of the between-study variability in the association between antiretroviral therapy and hypertriglyceridaemia, with the impact of antiretroviral therapy on the risk of hypercholesterolaemia being more than twice as high among HIV-infected patients with CD4 counts greater than 350 cells/mm^3 (Pooled OR 3.32, 95% CI 2.66 to 4.14), compared to HIV-infected patients with CD4 cell counts less than 350 cells/mm^3 (Pooled OR 1.44, 95% CI 1.03 to 2.01) (P = 0.02 for interaction).

Meta-regression analysis to determine factors that may potentially influence the association between antiretroviral therapy and the odds of dyslipidaemia was not performed because the number of observations required for this analysis were insufficient.

Table 8.7: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and the odds of hypertriglyceridaemia

Subgroup	N	Pooled OR (95% CI)	Meta-regression	
			P value	R^2 (%)
Region				
Sub-Saharan Africa	11	1.43 (1.11 to 1.84)		
Europe	4	3.89 (2.61 to 5.79)		
South-East Asia	4	3.54 (1.90 to 6.58)		
America	2	3.27 (2.02 to 5.32)	0.217	3.1
Income group				
High	5	3.82 (2.82 to 5.14)		
Low/middle	16	1.80 (1.41 to 2.43)	0.692	0
Females				
< 50%	9	2.86 (1.84 to 4.45)		
≥ 50%	10	1.71 (1.23 to 2.36)	0.242	2.5
Age group				
< 40 years	15	2.33 (1.60 to 3.12)		
≥ 40 years	3	3.03 (2.07 to 4.38)	0.891	0
Smokers				
< 50%	8	2.06 (1.27 to 3.34)		
≥ 50%	2	3.72 (2.40 to 5.75)	0.711	0
CD4 count				
< 350 cells/mm^3	8	1.44 (1.03 to 2.01)		
≥ 350 cells/mm^3	7	3.32 (2.66 to 4.14)	0.020	30.9
ART regimen				
PI-based	6	2.99 (1.89 to 4.74)		
Non-PI-based	12	1.63 (1.18 to 2.27)	0.631	0
ART duration				
< 18 months	4	2.16 (0.71 to 6.59)		
≥ 18 months	13	2.15 (1.58 to 2.93)	0.080	13.7
Study design				
Cohort	5	2.20 (1.14 to 4.42)		
Cross-sectional	16	2.31 (1.67 to 3.09)	0.768	0
Year of publication				
2000–2009	9	2.83 (1.78 to 4.20)		
2010–2015	12	1.91 (1.27 to 2.73)	0.662	0

ART, antiretroviral therapy; N, number of studies; OR, odds ratio; PI, protease inhibitor; R^2, explained variability. HIV infection duration was reported in less than ten studies and was excluded from the meta-regression analyses.

CHAPTER NINE

RESULTS – THE IMPACT OF ANTIRETROVIRAL THERAPY ON FASTING BLOOD GLUCOSE LEVELS AND DIABETES RISK

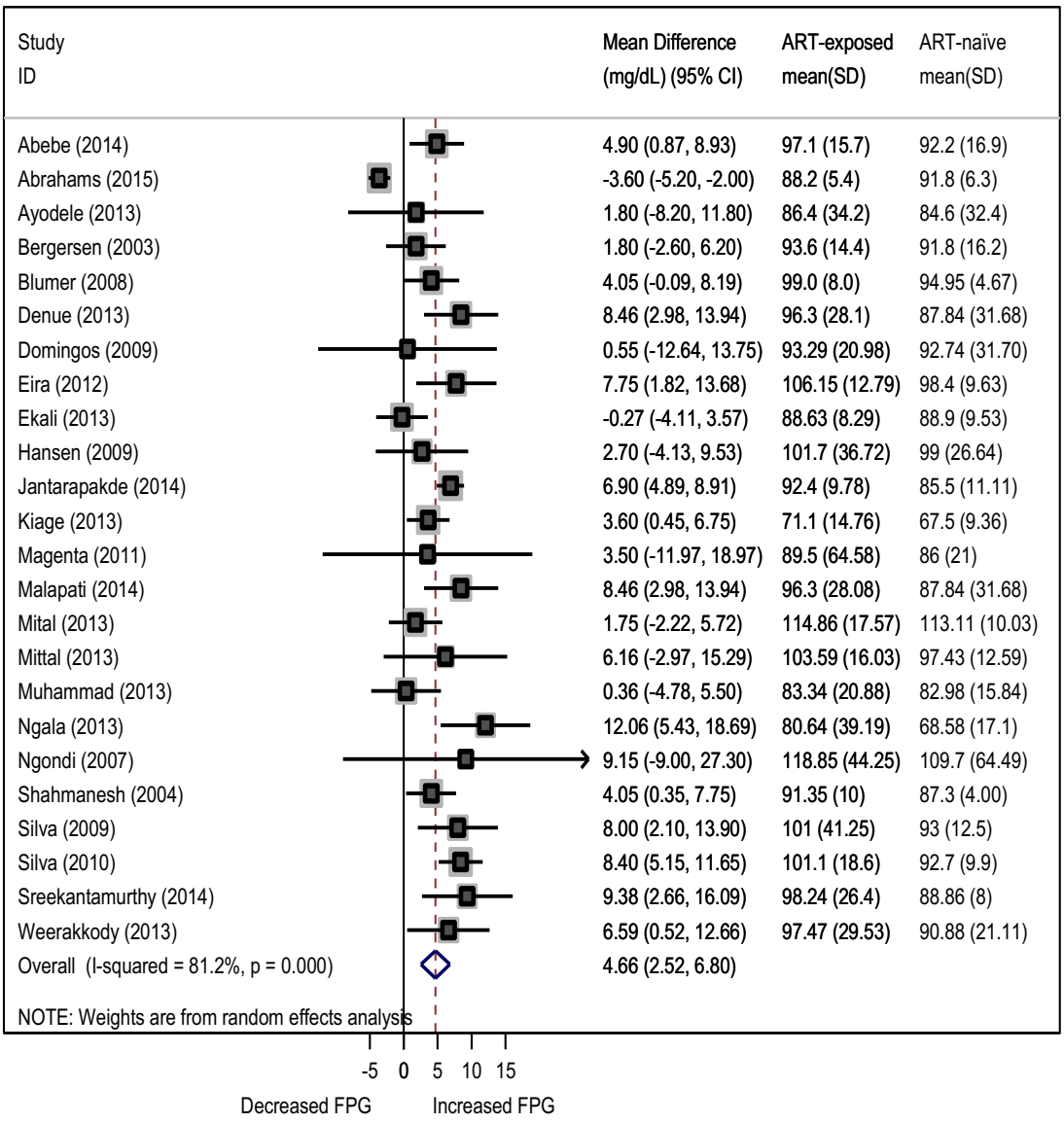
In this chapter, I present meta-analyses of the associations of antiretroviral therapy with fasting blood glucose concentrations and diabetes mellitus risk. I also identify study-level factors that may influence these pooled associations.

9.1 OVERALL ASSOCIATIONS OF ANTIRETROVIRAL THERAPY WITH FASTING BLOOD GLUCOSE CONCENTRATION AND DIABETES RISK

9.1.1 Increase in fasting blood glucose concentration

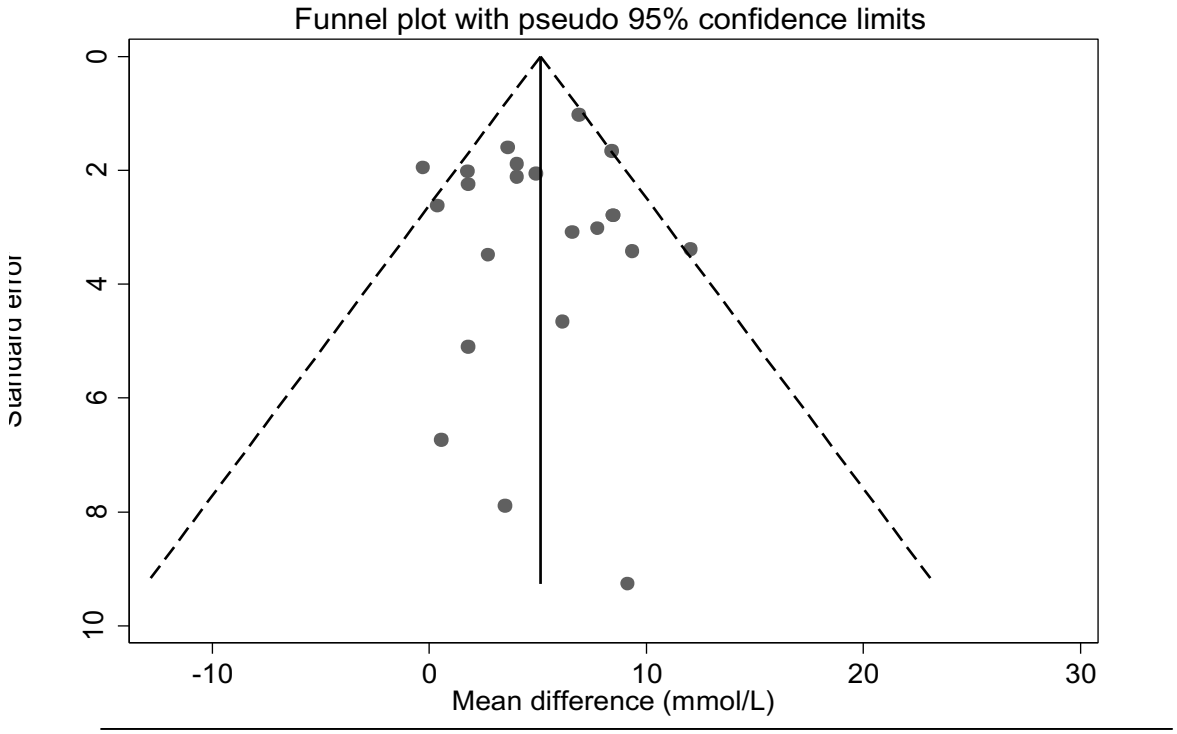
Mean fasting plasma glucose levels were compared between antiretroviral-exposed and antiretroviral-naïve patients in 24 studies, 12 of which reported significantly higher mean concentrations among antiretroviral-exposed patients (Abebe *et al*., 2014; Denue *et al*., 2013a; Eira *et al*., 2012; Jantarapakde *et al*., 2014; Kiage *et al*., 2013; Malapati *et al*., 2014; Ngala *et al*., 2013; Shahmanesh *et al*., 2004; Silva *et al*., 2009; Silva *et al*., 2010; Sreekantamurthy *et al*., 2014; Weerakkody *et al*., 2013) (Figure 9.1). Overall, mean fasting plasma glucose levels remained significantly higher in ART-exposed patients, compared to their naïve counterparts (Pooled MD 4.66 mg/dL, 95% CI 2.52 to 6.80). Heterogeneity across the included studies was considerable (I^2 = 81.2 %) and statistically significant ($P < 0.001$). Analysis of publication bias revealed a symmetrical funnel plot (Figure 9.2). Sensitivity analysis revealed no instance in which the sequential exclusion of studies altered the interpretation of the pooled association (Table 9.1).

Figure 9.1: Meta-analysis of the association between antiretroviral therapy and blood glucose level



ART, antiretroviral therapy; CI, confidence interval; FBG, fasting blood glucose. The black boxes and horizontal lines represent the mean difference in FBG with 95% CI in each study. The dashed vertical line through the diamond represents the pooled mean difference, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled mean difference. The solid vertical line through zero represents no effect.

Figure 9.2: Analysis of publication bias in the association of antiretroviral therapy with increasing fasting blood glucose levels

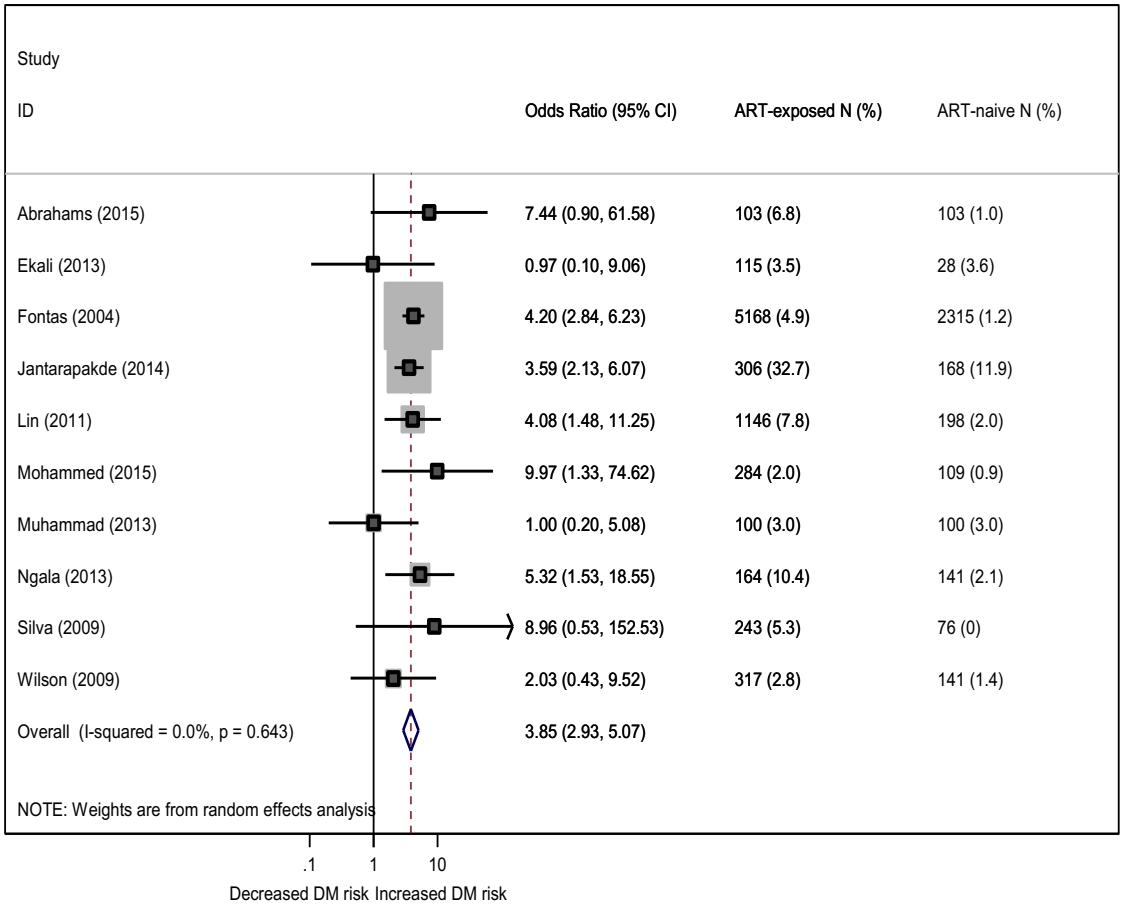


P-value for Egger's regression asymmetry test = 0·789

9.1.2 Increase in diabetes mellitus risk

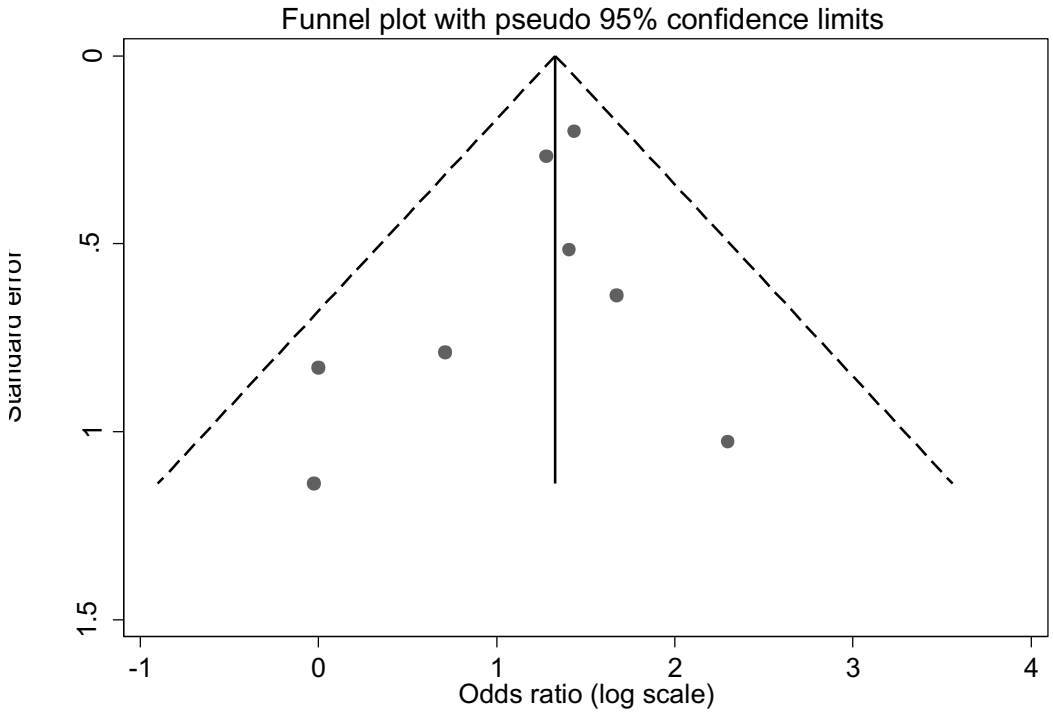
The prevalence estimates of diabetes were compared between antiretroviral-exposed and antiretroviral-naïve patients in ten studies, five of which revealed significantly higher odds of diabetes in the antiretroviral-exposed group (Fontas *et al*., 2004; Lin *et al*., 2011; Ngala *et al*., 2013; Jantarapakde *et al*., 2014; Mohammed *et al*., 2015). Among 7,946 participants with reported diabetes status in the antiretroviral-exposed groups, 519 (6.5%) had diabetes, as opposed to 63 of 3,379 (1.9%) in the antiretroviral-naïve groups. Overall, the odds ratios of diabetes were approximately four times higher among antiretroviral-exposed patients, compared to their naïve counterparts (Pooled OR 3.85, 95% CI 2.93 to 5.07) (Figure 9.3). Studies included in the meta-analysis were not heterogeneous (I^2 = 0%). No evidence of publication bias was found (P = 0.31 for Egger's test for funnel plot asymmetry) (Figure 9.4). Sensitivity analysis revealed no instance in which the sequential exclusion of studies altered the interpretation of the pooled association (Table 9.1).

Figure 9.3: Meta-analysis of the association between antiretroviral therapy and the odds of diabetes mellitus



ART, antiretroviral therapy; CI, confidence interval. The grey boxes and horizontal lines represent the odds ratios of dyslipidaemia with 95% CI in each study. The dashed vertical line through the diamond represents the pooled odds ratio, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled odds ratio. The solid vertical line through zero represents no effect.

Figure 9.4: Analysis of publication bias in the association between antiretroviral therapy and increased diabetes risk



P value for Egger's regression asymmetry test = 0.310

Table 9.1: Sensitivity analysis of the pooled associations of antiretroviral therapy with increased blood glucose levels and diabetes risk

Cardio-metabolic outcomes	Pooled estimate prior to serial exclusion of individual studies Pooled ES (95% CI)	Range of pooled estimate following serial exclusion of individual studies	Instance in which exclusion of a study changed interpretation of the pooled association Pooled ES (95% CI)
Fasting blood glucose	4.66 (2.52 to 6.80)	4.49 to 5.08	None
Diabetes mellitus	3.85 (2.93 to 5.07)	3.81 to 3.85	None

CI, confidence interval; Pooled ES, Pooled estimate

9.2 FACTORS THAT MAY INFLUENCE THE EFFECT OF ANTIRETROVIRAL THERAPY ON BLOOD GLUCOSE LEVEL

Table 9.2 presents the results of subgroup and meta-regression analyses. With the exception of antiretroviral treatment duration, the association of antiretroviral therapy with increased blood glucose concentration was significant across subgroups of all study-level characteristics. In addition, no statistically significant difference was observed between study-level subgroup estimates of the pooled association of antiretroviral therapy with increased fasting plasma glucose concentration. Antiretroviral therapy was significantly associated with increased blood glucose levels in studies where the mean antiretroviral treatment duration was at least 18 months (Pooled MD 4.97 mg/dL, 95% CI 3.10 to 6.84), whereas no significant association was observed in studies where the mean antiretroviral treatment duration was less than 18 months (Pooled MD 4.40 mg/dL, 95% CI -0.59 to 9.38) ($P = 0.615$ for interaction). Meta-regression analysis to determine factors that may potentially influence the association between antiretroviral therapy and the odds of diabetes mellitus was not performed because the number of observations required for this analysis were insufficient.

Table 9.2: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and blood glucose level

Subgroup	N	Pooled MD (95% CI)	Meta-regression	
			P-value	R^2 (%)
Region				
Sub-Saharan Africa	9	3.29 (0.48 to 7.06)		
Europe	5	3.34 (1.15 to 5.53)		
South-East Asia	5	5.89 (2.67 to 9.10)		
The Americas	4	7.92 (5.40 to 10.44)		
Western Pacific	1	6.90 (4.89 to 8.91)	0.313	3.1
Income group				
High income	5	3.34 (1.15 to 5.53)		
Low/middle	19	5.07 (2.45 to 7.69)	0.469	3
Females				
≤ 50%	10	5.69 (3.86 to 7.51)		
> 50%	10	3.80 (0.04 to 7.56)	0.341	0
Age group				
< 40 years	13	4.46 (1.43 to 7.49)		
≥ 40 years	9	5.44 (3.39 to 7.49)	0.502	0
Smokers				
≤ 50%	11	5.17 (3.43 to 6.91)		
> 50%	3	4.13 (0.04 to 8.29)	0.692	0
CD4 cell count				
< 350 cells/mm^3	10	4.10 (1.91 to 6.30)		
≥ 350 cells/mm^3	7	5.62 (3.89 to 7.34)	0.371	12.4
HIV duration				
< 60 months	6	5.63 (3.10 to 8.15)		
≥ 60 months	6	4.83 (2.00 to 7.67)	0.714	0
ART regimen				
PI-based	15	4.45 (1.62 to 7.27)		
Non-PI-based	5	3.79 (0.66 to 6.93)	0.839	0
ART duration				
< 18 months	7	4.40 (-0.59 to 9.38)		
≥ 18 months	14	4.97 (3.10 to 6.84)	0.615	0
Study design				
Cohort	7	4.11 (-0.75 to 8.96)		
Cross-sectional	17	4.93 (3.23 to 6.62)	0.479	0
Selection bias				
High/unclear	19	4.61 (2.08 to 7.14)		
Low	5	3.70 (1.04 to 6.36)	0.832	0

ART, antiretroviral therapy; MD, mean difference; N, number of studies; PI, protease inhibitor; R^2, explained variability

CHAPTER TEN

RESULTS – THE IMPACT OF ANTIRETROVIRAL THERAPY ON BODY FAT MEASURES

In this chapter, I present meta-analyses of the associations of antiretroviral therapy with different measures of body fat, including body mass index and waist circumference. I also present meta-analyses of the associations of antiretroviral therapy with combined overweight/obesity and central obesity, and identify study-level factors that may influence these pooled associations.

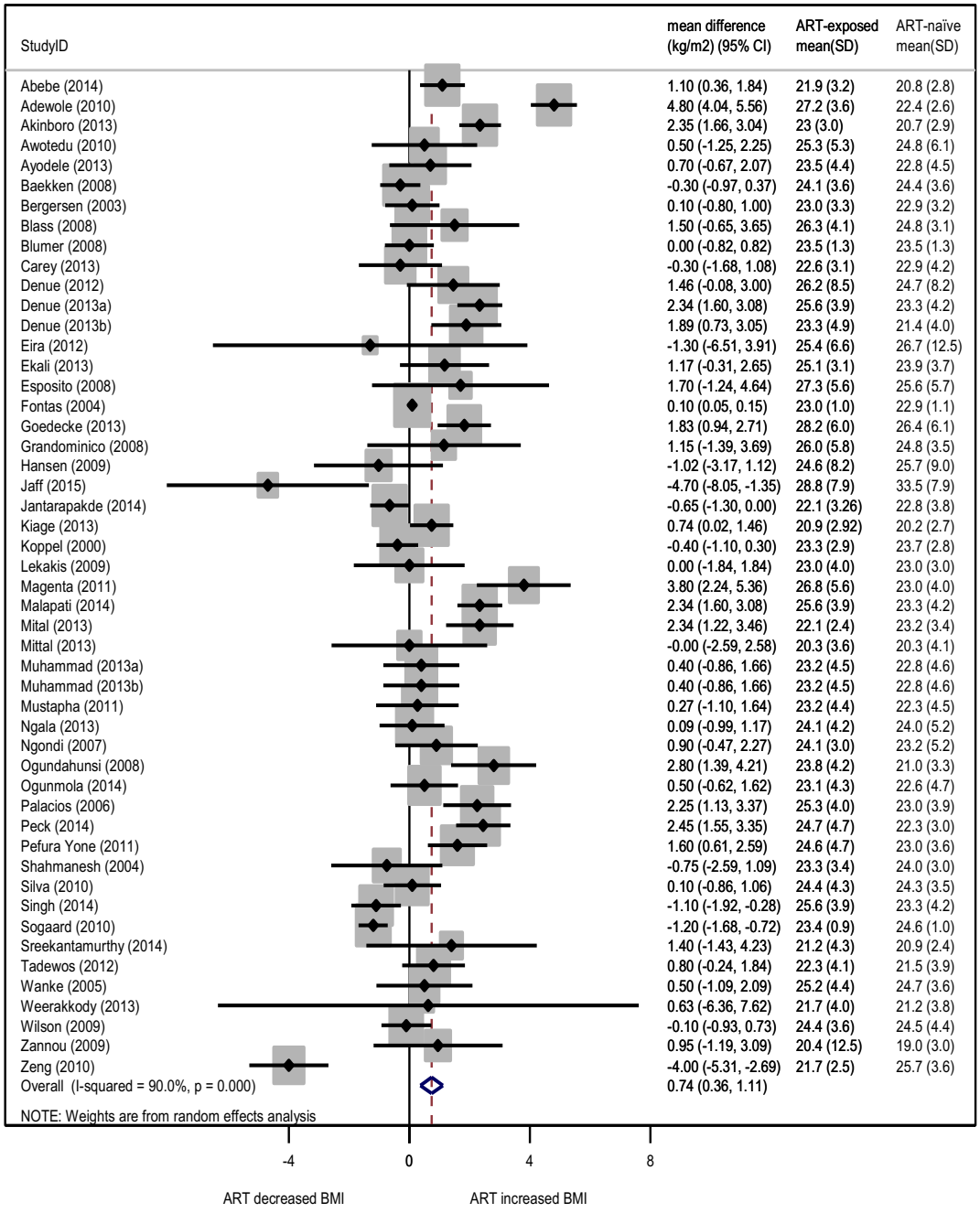
10.1 OVERALL ASSOCIATIONS OF ANTIRETROVIRAL THERAPY WITH DIFFERENT MEASURES OF BODY FAT

10.1.1 Increase in body mass index

Mean body mass index was compared between antiretroviral-exposed and antiretroviral-naïve patients in 50 studies. Thirty seven of these studies reported larger body mass indices among patients on antiretroviral therapy (Figure 10.1). Overall, the mean body mass index among patients on antiretroviral therapy was significantly higher, compared to that among patients who were naïve to antiretroviral therapy (Pooled MD 0.74 kg/m^2, 95% CI 0.36 to 1.11, 17,041 participants). Heterogeneity across the 50 studies was statistically significant ($P < 0.001$ for Chi^2 test for heterogeneity) and explained 90% of variability in the mean differences between individual studies. Although funnel plot revealed an underrepresentation of smaller studies ($P = 0.02$ for Egger's regression test for funnel plot asymmetry), there was no observed tendency for larger studies to report a significant association between antiretroviral therapy and body mass index (Figure 10.2). Moreover, correction for publication bias using the trim and fill analysis did not alter the

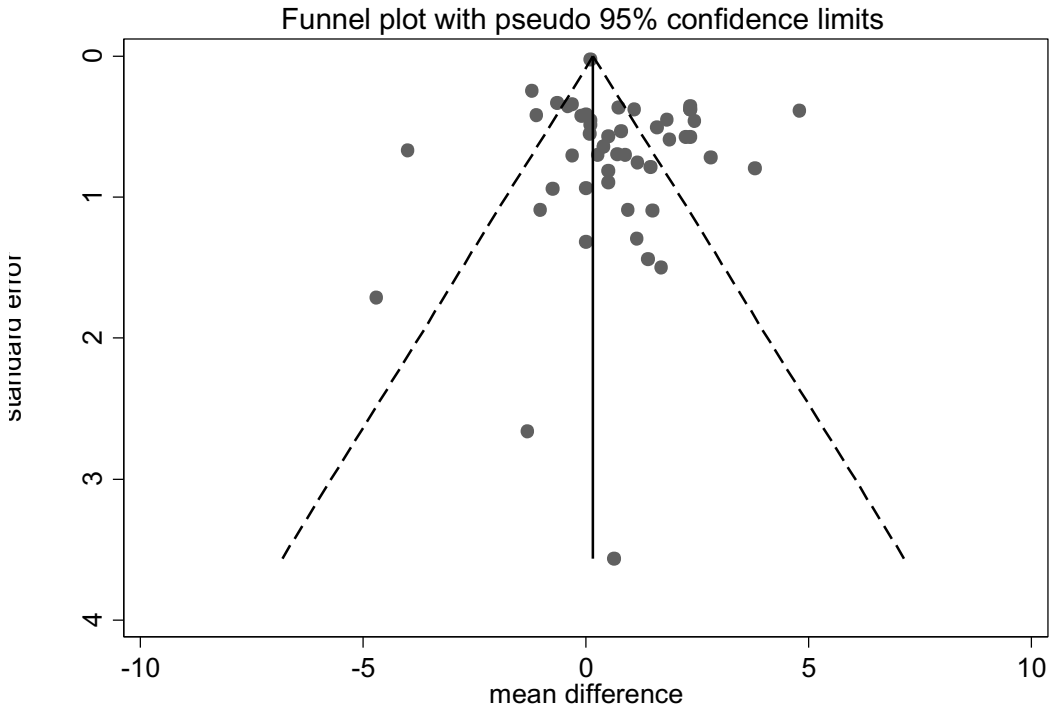
overall interpretation of the pooled association as the filled dataset remained unchanged from the original dataset (Figure 10.3). Hence, publication bias did not have a significant impact of the pooled association between antiretroviral therapy and body mass index. Sensitivity analysis revealed no indication that any of the included studies had undue influence on the pooled estimate as to alter the interpretation of the association (Table 10.1).

Figure 10.1: Meta-analysis of the association between antiretroviral therapy and body mass index



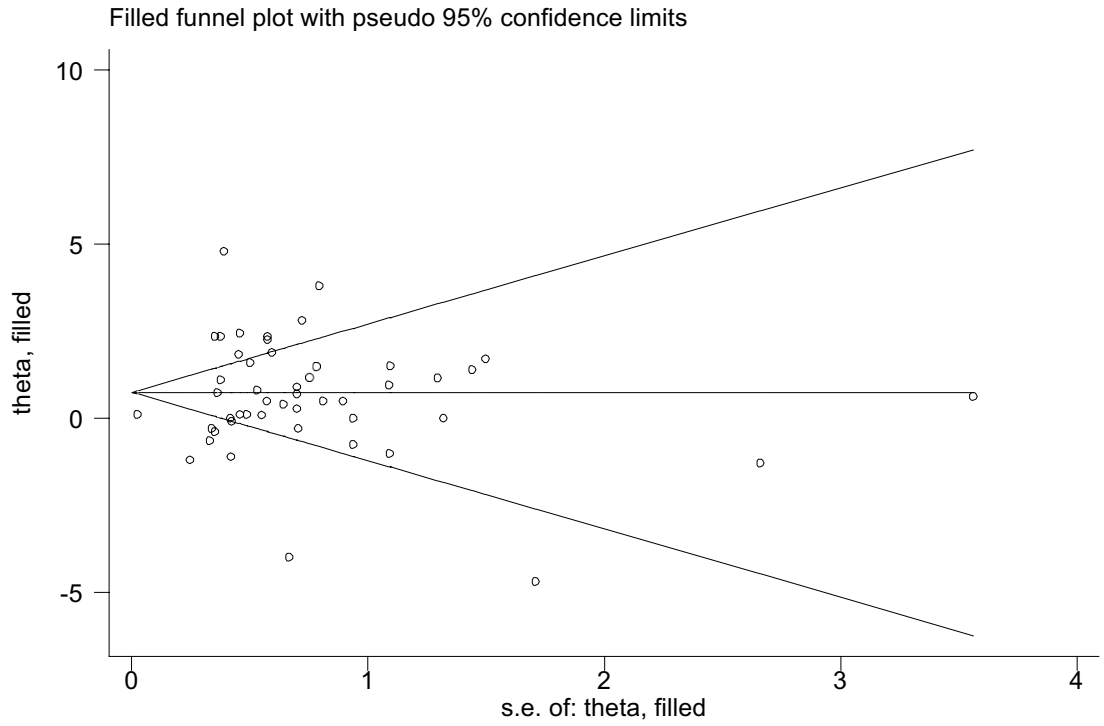
ART, antiretroviral therapy; BMI, body mass index; CI, confidence interval. The grey boxes and horizontal lines represent the mean difference with 95% CI for each study. The dashed vertical line through the diamond represents the pooled mean difference, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled mean difference. The solid vertical line through zero represents no effect.

Figure 10.2: Analysis of publication bias in the association between antiretroviral therapy and body mass index



Funnel plot showing an underrepresentation of smaller studies towards the base of the graph. $P = 0.02$ for Egger's regression test for funnel plot asymmetry.

Figure 10.3: Filled analysis of publication bias in the association between antiretroviral therapy and body mass index

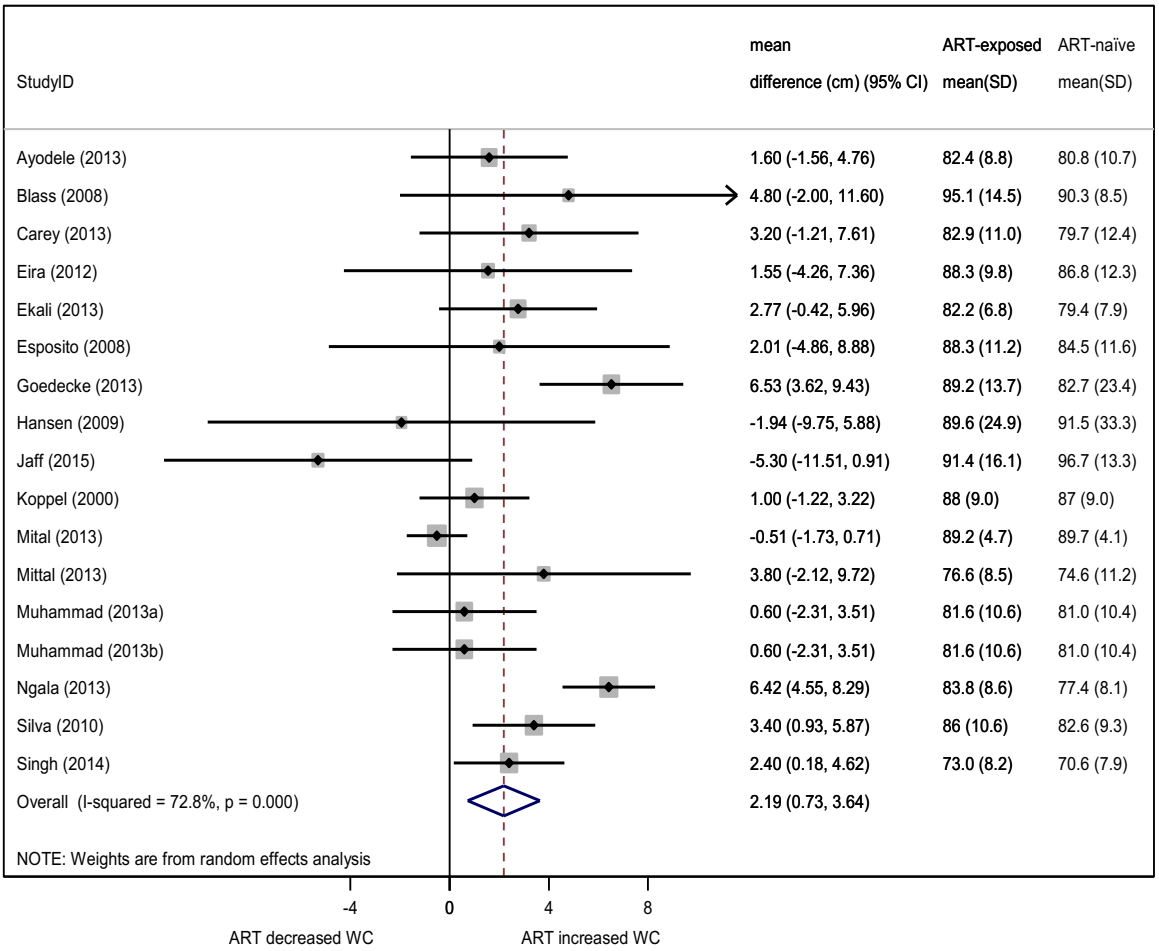


Trim and fill analysis of publication bias revealed no additional studies imputed to the original dataset. Theta stands for the mean difference.

10.1.2 Increase in waist circumference

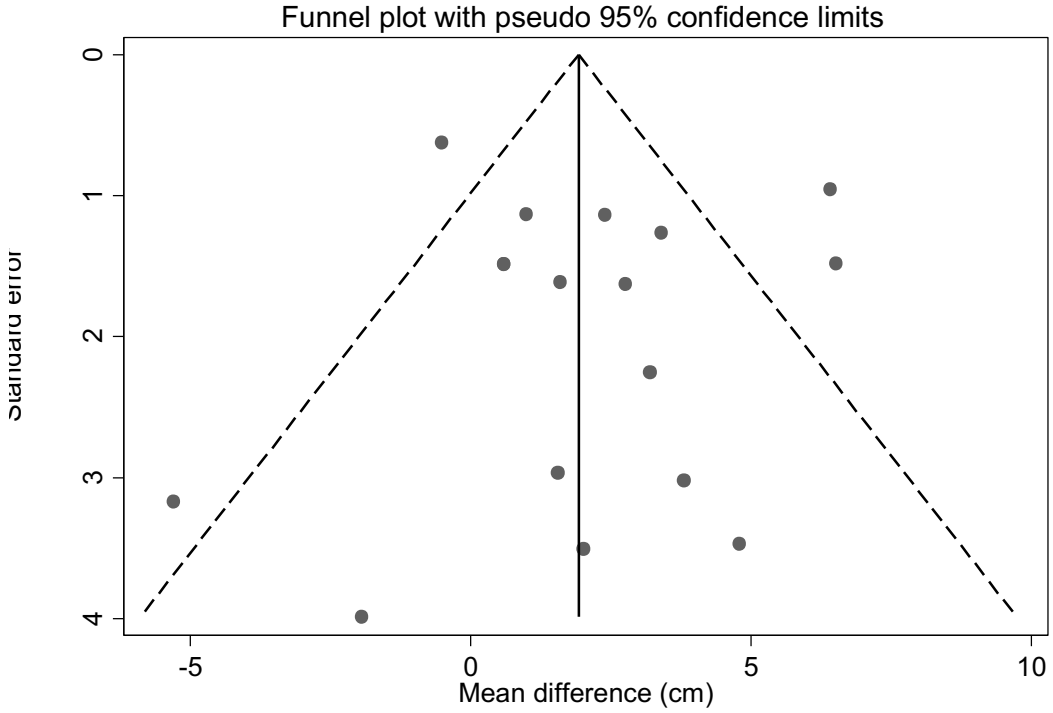
Seventeen studies compared waist circumference measurements between antiretroviral-exposed and naïve patients. Fourteen of these studies reported a larger mean waist circumference in patients on antiretroviral therapy, compared to treatment-naïve patients. Overall, the mean waist circumference of antiretroviral-exposed patients was significantly larger in comparison to that for patients who were naïve to antiretroviral therapy (Pooled MD 2.19 cm, 95% CI 0.73 to 3.64, 3641 participants) (Figure 10.4). Heterogeneity was statistically significant across all 17 studies ($P < 0.001$ for Chi^2 test for heterogeneity) and explained a considerable amount of between-study variability in individual mean differences ($I^2 = 72.8\%$). Funnel plot asymmetry was absent ($P = 0.65$ for Egger's regression test), suggesting no evidence of publication bias (Figure 10.5). Sensitivity analysis revealed no indication that any of the included studies had undue influence on the pooled estimate as to alter the interpretation of the association (Table 10.1).

Figure 10.4: Meta-analysis of the association between antiretroviral therapy and waist circumference



ART, antiretroviral therapy; CI, confidence interval; WC, waist circumference. The grey boxes and horizontal lines represent the mean difference with 95% CI for each study. The dashed vertical line through the diamond represents the pooled mean difference, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled mean difference. The solid vertical line through zero represents no effect.

Figure 10.5: Analysis of publication bias in the association between antiretroviral therapy and waist circumference

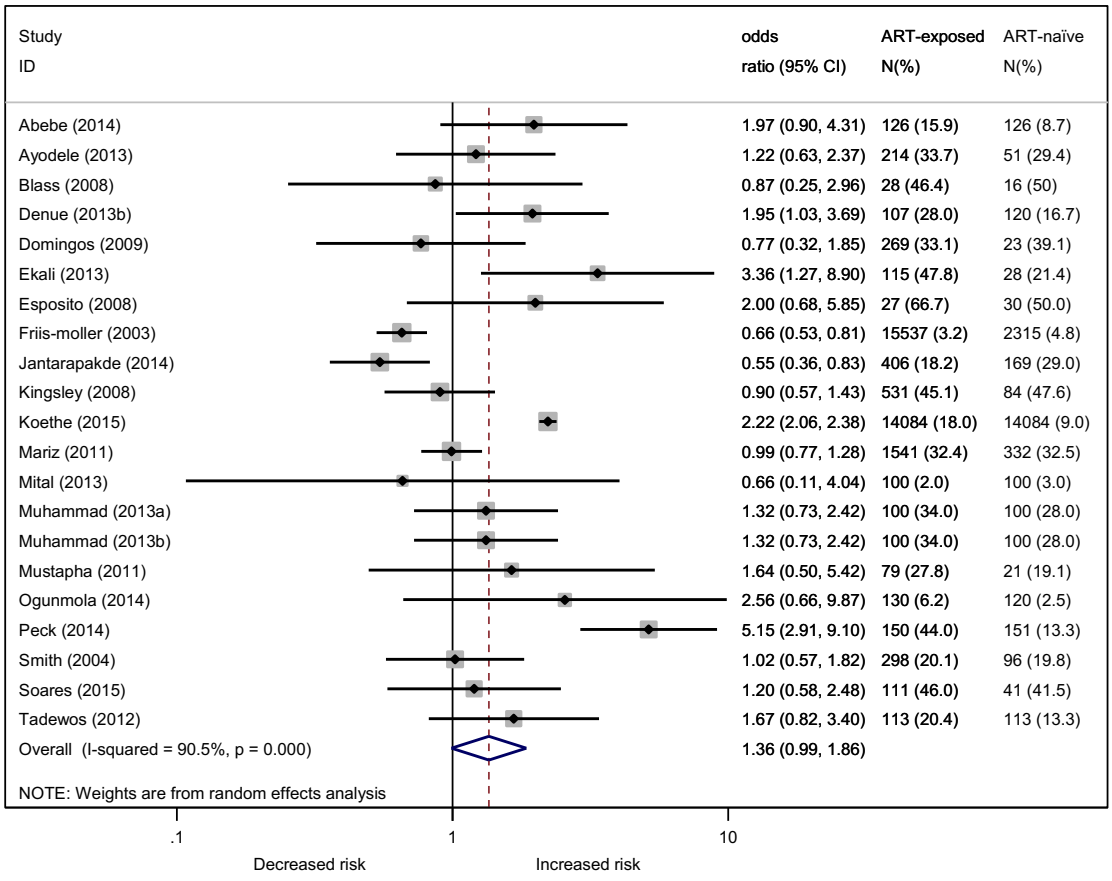


P = 0.65 for Egger's test for small study effects

10.1.3 Increase in the risk of overweight/obesity

Prevalence estimates of combined overweight/obesity were compared between antiretroviral-exposed and antiretroviral-naïve patients in 21 studies. Although 13% (4441 of 34,166) of patients on antiretroviral therapy were either overweight or obese, compared to 9.9% (1,797 of 18,220) in the antiretroviral-naïve group, the difference was not statistically significant (pooled OR 1.36, 95% CI 0.99 to 1.86) (Figure 33). Sensitivity analysis revealed no indication that any of the included studies had undue influence on the pooled estimate as to alter the interpretation of the association (Table 10.1).

Figure 10.6: Meta-analysis of the association between antiretroviral therapy and combined overweight/obesity

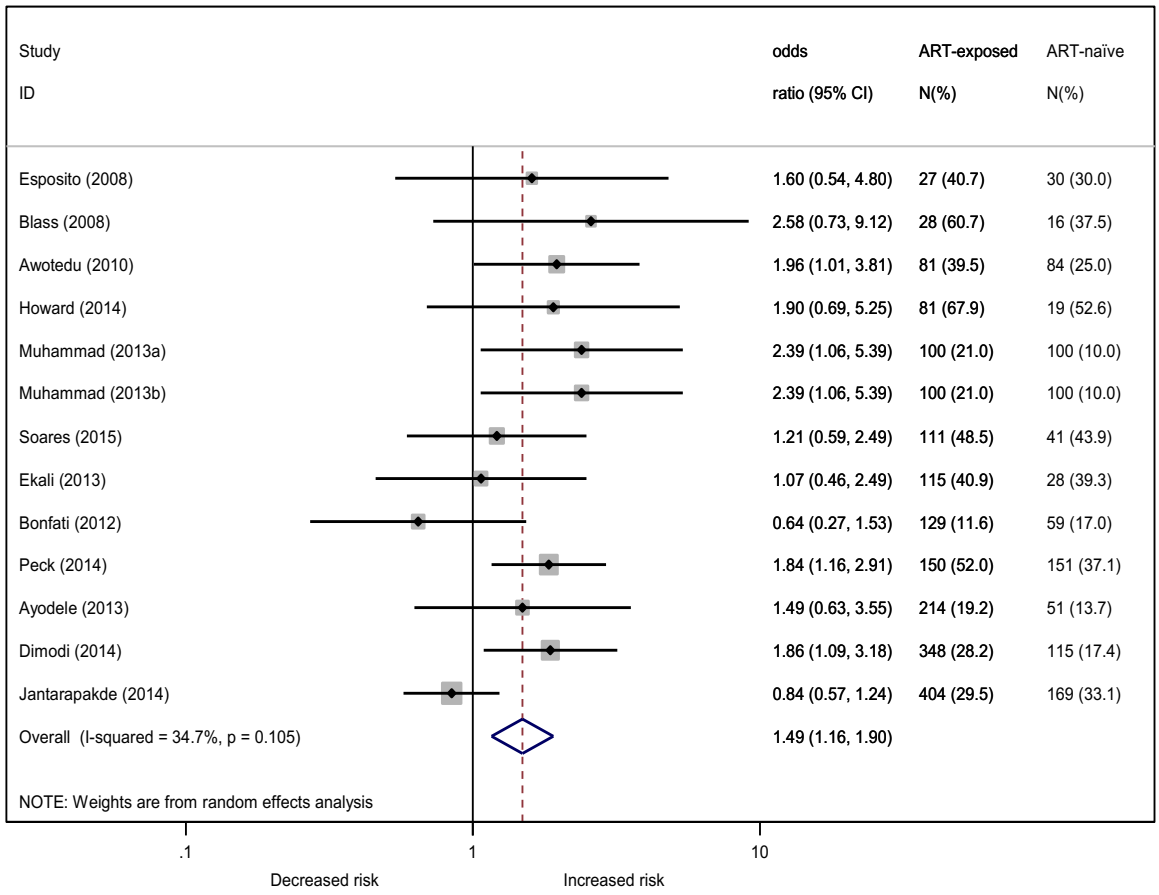


ART, antiretroviral therapy; CI, confidence interval. The grey boxes and horizontal lines represent the odds ratios of overweight/obesity with 95% CI in each study. The interrupted vertical line through the diamond represents the pooled odds ratio, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled odds ratio. The solid vertical line through 1 represents no effect.

10.1.4 Increase in the risk of central obesity

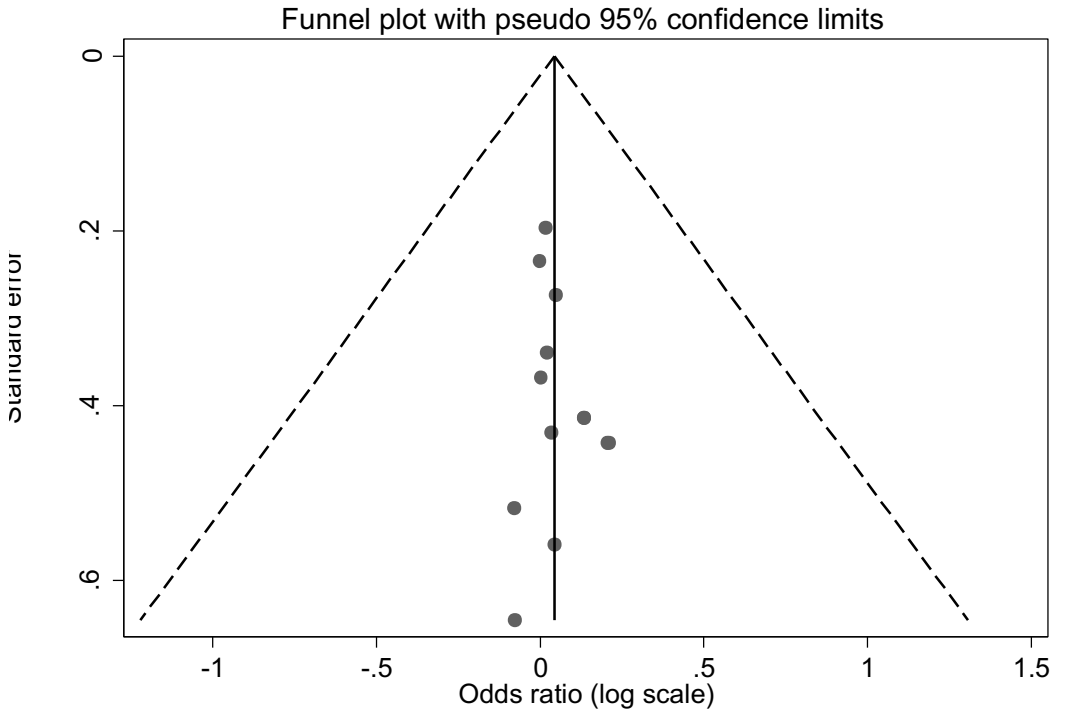
Eleven of the 13 studies that compared prevalence estimates of central obesity between antiretroviral-exposed and antiretroviral-naïve patients reported higher proportions in the former. Overall, 609 (32.3%) of 1888 HIV-infected patients on antiretroviral therapy were centrally obese, compared to 244 (25.3%) of 963 antiretroviral-naïve patients (Pooled OR 1.49; 95% CI 1.16 to 1.90) (Figure 10.7). Heterogeneity across all 13 studies was not statistically significant and only accounted for a small amount of between-study variability (I^2 = 34.7%). No evidence of publication bias was found (P = 0.35 for Egger's test for small-study effects) (Figure 10.8). Sensitivity analysis revealed no indication that any of the included studies had undue influence on the pooled estimate as to alter the interpretation of the association (Table 10.1).

Figure 10.7: Meta-analysis of the association between antiretroviral therapy and central obesity



ART, antiretroviral therapy; CI, confidence interval. The grey boxes and horizontal lines represent the odds ratios of central obesity with 95% CI in each study. The dashed vertical line through the diamond represents the pooled odds ratio, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled odds ratio. The solid vertical line through 1 represents no effect.

Figure 10.8: Analysis of publication bias in the association between antiretroviral therapy and central obesity



P = 0.35 for Egger's test for small study effects

Table 10.1: Sensitivity analysis of the pooled associations of antiretroviral therapy with body mass index and waist circumference

Cardio-metabolic outcomes	Pooled estimate prior to serial exclusion of individual studies	Range of pooled estimate following serial exclusion of individual studies	Instance in which exclusion of a study changed interpretation of the pooled association
	Pooled ES (95% CI)		Pooled ES (95% CI)
Body mass index	0.74 (0.36 to 1.11)	0.64 to 0.79	None
Waist circumference	2.19 (0.73 to 3.64)	2.13 to 2.22	None
Overweight/obesity	1.36 (0.99 to 1.86)	1.31 to 1.42	None
Central obesity	1.49 (1.16 to 2.27)	1.45 to 1.55	None

CI, confidence interval; Pooled ES, Pooled estimate;

10.2 FACTORS THAT MAY INFLUENCE THE EFFECTS OF ANTIRETROVIRAL THERAPY ON BODY MASS INDEX AND WAIST CIRCUMFERENCE

Table 10.2 presents results of the subgroup and meta-regression analyses of the association between antiretroviral therapy and body mass index. Estimates of the pooled association were significantly different between subgroups of geographical region, CD4 cell count, antiretroviral regimen and study design. Body mass indices were significantly higher among antiretroviral-exposed patients than their naïve counterparts in studies conducted in sub-Saharan African countries (Pooled MD 1.36 kg/m^2, 95% CI 0.81 to 1.91), but not in studies conducted in countries of other geographical regions (P = 0.002 for interaction). Antiretroviral therapy was associated with a statistically significant increase in body mass index among HIV-infected patients with CD4 counts below 350 cells/mm^3 (Pooled MD 1.53 kg/m^2, 95% CI 1.10 to 1.96), whereas no statistically significant association was observed among HIV-infected patients with CD4 counts greater than 350 cells/mm^3 (Pooled MD 0.04 kg/m^2, 95% CI -0.43 to 0.51) (P = 0.001 for interaction). Antiretroviral therapy was also associated with significant increase in body mass index among HIV-infected patients on non-protease inhibitor-based antiretroviral treatment regimens (Pooled MD 1.43 kg/m^2, 95% CI 0.82 to 2.05), but not among HIV-infected patients on protease inhibitors (Pooled MD 0.44 kg/m^2, 95% CI -0.18 to 1.06) (P = 0.049 for interaction). Antiretroviral therapy was associated with more than two times the increase in body mass index among HIV infected patients on (antiretroviral) treatment for less than 18 months (Pooled MD 1.59 kg/m^2, 95% CI 0.78 to 2.41), compared to HIV-infected patients who have received treatment for more than 18 months (Pooled MD 0.60 kg/m^2, 95% CI 0.14 to 1.06) (P = 0.047 for interaction). Cohort studies were more likely to report statistically significant associations between antiretroviral therapy and increased

body mass indices (Pooled MD 1.48 kg/m^2, 95% CI 0.77 to 2.19), compared to cross-sectional studies (Pooled MD 0.25 kg/m^2, 95% CI -0.23 to 0.74) (P = 0.020 for interaction). The differences between studies in mean CD4 cell counts explained most of the variability between studies. No statistically significant differences in the pooled association were observed between subgroups of other study-level characteristics.

Table 10.2: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and body mass index

Subgroup	N	Pooled MD (95% CI)	Meta-regression	
			P value	R^2 (%)
Region				
Sub-Saharan Africa	24	1.36 (0.81 to 1.91)		
Europe	13	0.17 (-0.32 to 0.67)		
South-East Asia	8	0.44 (-0.77 to 1.66)		
The Americas	4	0.26 (-0.51 to 1.03)		
Western Pacific	1	-4.00 (-5.31 to -2.69)	0.002	23.9
Income group				
High income	15	0.21 (-0.26 to 0.68)		
Low/middle	35	0.90 (0.33 to 1.47)	0.216	3.3
Females				
< 50%	21	0.22 (-0.28 to 0.71)		
≥ 50%	23	1.11 (0.48 to 1.73)	0.070	9.8
Age group				
< 40 years	30	0.66 (0.26 to 1.07)		
≥ 40 years	18	0.67 (-0.11 to 1.45)	0.937	0
Smokers				
< 50%	18	0.72 (-0.16 to 1.60)		
≥ 50%	7	0.38 (-1.67 to 2.43)	0.726	0
CD4 count				
< 350 cells/mm^3	22	1.53 (1.10 to 1.96)		
≥ 350 cells/mm^3	18	0.04 (-0.43 to 0.51)	0.001	34.5
HIV duration				
< 60 months	8	0.49 (-0.41 to 1.40)		
≥ 60 months	9	-0.22 (-0.55 to 0.11)	0.199	7.3
ART regimen				
PI-based	18	0.44 (-0.18 to 1.06)		
Non-PI-based	22	1.43 (0.82 to 2.05)	0.049	9.1
ART duration				
< 18 months	14	1.59 (0.78 to 2.41)		
≥ 18 months	28	0.60 (0.14 to 1.06)	0.047	9.6
Study design				
Cohort	19	1.48 (0.77 to 2.19)		
Cross-sectional	31	0.25 (-0.23 to 0.74)	0.006	13.4
Selection bias				
Low risk	16	0.24 (0.03 to 0.45)		
High/unclear risk	34	0.13 (0.01 to 0.24)	0.384	0
Publication year				
2000–2009	17	0.36 (-0.03 to 0.74)		
2010–2014	33	0.81 (0.18 to 1.44)	0.492	0

ART, antiretroviral therapy; MD, mean difference; N, number of studies; PI, protease inhibitor; R^2, explained variability.

Table 10.3 presents the results of the subgroup and meta-regression analyses of the association between antiretroviral therapy and waist circumference. While these results showed no evidence of a statistically significant differential between subgroup estimates of the pooled association, meta-regression analyses revealed that differences among studies in mean age (R^2 = 18.5%), mean CD4 cell count (R^2 = 8.0%), antiretroviral treatment regimen (R^2 = 8.2%), and mean duration of antiretroviral therapy (R^2 = 4.2%) accounted for some of the between-study variability in the association between antiretroviral therapy and waist circumference. Of note, meta-regression analyses were not performed on the proportions of smokers and the mean duration of HIV because these study-level factors were reported in fewer than 10 studies.

Table 10.3: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and waist circumference

Subgroup	N	Pooled MD (95% CI)	Meta-regression	
			P value	R^2 (%)
Region				
Sub-Saharan Africa	8	2.56 (0.19 to 4.92)		
Europe	3	1.14 (-0.89 to 3.18)		
South-East Asia	4	1.25 (-0.92 to 3.42)		
The Americas	2	3.12 (0.84 to 5.39)	0.679	0
Income group				
High income	3	1.14 (-0.89 to 3.18)		
Low/middle	14	2.31 (0.66 to 3.96)	0.615	0
Females				
< 50%	6	2.16 (0.90 to 3.43)		
≥ 50%	9	2.64 (0.49 to 4.80)	0.681	0
Age group				
< 40 years	11	3.16 (1.72 to 4.60)		
≥ 40 years	5	0.36 (-2.32 to 3.03)	0.092	18.5
CD4 count				
< 350 cells/mm^3	7	1.75 (0.55 to 2.95)		
≥ 350 cells/mm^3	5	3.19 (0.60 to 5.77)	0.253	8.0
ART regimen				
PI-based	4	3.96 (0.40 to 7.66)		
Non-PI-based	8	2.13 (0.05 to 4.21)	0.358	8.2
ART duration				
< 18 months	4	3.67 (1.00 to 6.35)		
≥ 18 months	11	2.40 (1.07 to 3.72)	0.338	4.2
Study design				
Cohort	2	2.57 (0.49 to 4.65)		
Cross-sectional	15	2.08 (0.44 to 3.72)	0.751	0
Selection bias				
Low risk	2	0.30 (-0.02 to 0.63)		
High/unclear risk	15	0.19 (0.05 to 0.33)	0.620	0
Publication year				
2000–2009	4	1.42 (-0.50 to 3.35)		
2010–2014	12	2.24 (0.52 to 3.95)	0.806	0

ART, antiretroviral therapy; MD, mean difference; N, number of studies; PI, protease inhibitor; R^2, explained variability. HIV duration was excluded from meta-regression analyses because less than 10 studies reported the mean duration of HIV infection.

Pooled estimates of the association between antiretroviral therapy and combined overweight/obesity were not significantly different between study-level subgroups (Table 10.4). However, estimates for studies conducted in sub-Saharan Africa (Pooled OR 1.96, 95% CI 1.44 to 2.67), studies in which the mean age of the participants was 40 years or more (Pooled OR 1.77, 95% CI 1.01 to 3.11), studies in which the mean CD4 cell count was less than 350 cells/mm^3 (pooled OR 2.00, 95% CI 1.28 to 3.14), studies with more women than men (Pooled OR 1.73, 95% CI 1.11 to 2.71), studies in which all antiretroviral-exposed participants received non-protease inhibitor-based antiretroviral regimens (Pooled OR 1.60, 95% CI 1.25 to 2.04), and studies in which the mean duration of antiretroviral therapy was less than 18 months (Pooled OR 1.62, 95% CI 1.06 to 2.47) indicated higher odds of combined overweight/obesity for antiretroviral-exposed patients than their naive counterparts. Conversely, subgroups such as studies conducted outside the sub-Saharan African region, mean age < 40 years, fewer women than men, mean CD4 cell count ≥ 350 cells/mm^3, and protease inhibitors (Pooled OR 1.04, 95% CI 0.54 to 1.99), did not show any statistically significant association between antiretroviral therapy and combined overweight/obesity. CD4 count differences across studies accounted for the majority of between-study variability in the association between antiretroviral therapy and the odds of overweight/obesity (R^2 = 16.2%). Meta-regression analyses could not be performed on smoking status because none of the included studies in these analyses comprised more smokers than non-smokers.

Table 10.4: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and combined overweight/obesity

Subgroup	N	Pooled OR (95% CI)	Meta-regression	
			P value	R^2 (%)
Region				
Sub-Saharan Africa	11	1.96 (1.44 to 2.67)		
Europe	3	0.71 (0.56 to 0.89)		
South-East Asia	2	0.55 (0.37 to 0.83)		
The Americas	5	1.12 (0.69 to 2.01)	0.061	12.8
Income group				
High income	5	1.05 (0.51 to 2.18)		
Low/middle	16	1.48 (1.07 to 2.03)	0.275	1.0
Females				
< 50%	8	1.03 (0.61 to 1.72)		
≥ 50%	11	1.73 (1.11 to 2.71)	0.061	14.3
Age group				
< 40 years	12	1.35 (0.95 to 1.94)		
≥ 40 years	5	1.77 (1.01 to 3.11)	0.453	0
CD4 count				
< 350 cells/mm^3	7	2.00 (1.28 to 3.14)		
≥ 350 cells/mm^3	8	1.01 (0.70 to 1.45)	0.093	16.2
ART regimen				
PI-based	6	1.04 (0.54 to 1.99)		
Non-PI-based	10	1.60 (1.25 to 2.04)	0.745	0
ART duration				
< 18 months	3	1.62 (1.06 to 2.47)		
≥ 18 months	11	1.31 (0.83 to 2.06)	0.971	0
Study design				
Cohort	4	1.50 (0.65 to 3.44)		
Cross-sectional	17	1.31 (0.98 to 1.75)	0.899	0
Selection bias				
Low risk	7	1.42 (0.81 to 2.49)		
High/unclear risk	14	1.27 (0.95 to 1.69)	0.543	0
Publication year				
2000–2009	6	0.81 (0.63 to 1.05)		
2010–2014	15	1.58 (1.14 to 2.18)	0.113	9.5

ART, antiretroviral therapy; OR, odds ratio; N, number of studies; PI, protease inhibitor; R^2, explained variability. Smoking status and HIV duration were excluded from meta-regression analyses because less than ten studies reported the proportions of smokers and the mean duration of HIV infection.

Table 10.5 presents results of the subgroup and meta-regression analyses of the association between antiretroviral therapy and the risk of central obesity. Regional variations in the pooled estimate accounted for the largest amount of variability between studies, with antiretroviral therapy associated with a statistically significant increase in the risk of central obesity in HIV-infected populations in sub-Saharan African countries (Pooled OR 1.82, 95% CI 1.43 to 2.32); whereas, no significant association was observed in the other regions ($P = 0.036$ for interaction, $R^2 = 63.7\%$). While there was no evidence of a statistically significant difference between other subgroup estimates of the pooled association, it is worth noting that the antiretroviral therapy was associated with a significant increase in the risk of central obesity in HIV-infected patients who were 40 years and above (Pooled OR 1.69, 95% CI 1.17 to 2.45), but not among HIV-infected patients who were less than 40 years old (Pooled OR 1.36, 95% CI 0.94 to 1.96). Likewise, studies with more women than men reported significant association between antiretroviral therapy and increased risk of central obesity (Pooled OR 1.57, 95% CI 1.17 to 2.09); whereas, studies with fewer women than men reported no significant association between antiretroviral therapy and central obesity (Pooled OR 1.14, 95% CI 0.58 to 2.23). Antiretroviral therapy was also associated with an increased risk of central obesity among HIV-infected patients with CD4 cell counts less than 350 cells/mm^3 (Pooled OR 1.78, 95% CI 1.32 to 2.40); whereas, no significant association was observed among HIV-infected patients with CD4 cell counts greater than 350 cells/mm^3 (Pooled OR 1.09, 95% CI 0.66 to 1.80). While the difference between these subgroup estimates were not statistically significant ($P = 0.221$ for interaction), it accounted for 36.9% of between-study variability in the effect estimates. Subgroup and meta-regression analyses of the association between antiretroviral therapy and central obesity could not be performed on smoking status because none of the studies included

in these analyses comprised more smokers than non-smokers, so that comparison with studies comprising fewer smokers than non-smokers was not possible. Similarly, the mean duration of HIV infection was greater than 60 months in all of the included studies, so that subgroup and meta-regression analyses could not be performed on HIV-infection duration.

Table 10.5: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and the odds of central obesity

Subgroup	N	Pooled OR (95% CI)	Meta-regression	
			P value	R^2 (%)
Region				
Sub-Saharan Africa	8	1.82 (1.43 to 2.32)		
Europe	3	1.36 (0.57 to 3.21)		
South-East Asia	1	0.84 (0.57 to 1.24)		
The Americas	1	1.21 (0.59 to 2.49)	0.036	63.7
Income group				
High income	3	1.52 (1.17 to 1.97)		
Low/middle	10	1.36 (0.57 to 3.21)	0.886	0
Females				
< 50%	3	1.14 (0.58 to 2.23)		
≥ 50%	9	1.57 (1.17 to 2.09)	0.425	0
Age group				
< 40 years	8	1.36 (0.94 to 1.96)		
≥ 40 years	3	1.69 (1.17 to 2.45)	0.602	0
CD4 count				
< 350 cells/mm^3	4	1.78 (1.32 to 2.40)		
≥ 350 cells/mm^3	6	1.09 (0.66 to 1.80)	0.221	36.9
ART regimen				
PI-based	6	1.37 (0.89 to 2.10)		
Non-PI-based	5	1.74 (1.18 to 2.57)	0.501	0
ART duration				
< 18 months	2	1.53 (0.78 to 3.03)		
≥ 18 months	8	1.37 (0.95 to 1.97)	0.975	0
Study design				
Cohort	2	0.95 (0.39 to 2.31)		
Cross-sectional	11	1.56 (1.21 to 2.01)	0.224	5.8
Selection bias				
Low risk	4	1.51 (0.87 to 2.62)		
High/unclear risk	9	1.48 (1.10 to 1.98)	0.971	0
Year of publication				
2000–2009	9	1.97 (0.86 to 4.50)		
2010–2014	4	1.46 (1.12 to 1.91)	0.885	0

ART, antiretroviral therapy; OR, odds ratio; N, number of studies; PI, protease inhibitor; R^2, explained variability. Smoking status, CD4 count and HIV duration were excluded from meta-regression analyses because less than ten studies reported the proportions of smokers, mean CD4 count, and mean duration of HIV infection.

CHAPTER ELEVEN

RESULTS – THE IMPACT OF ANTIRETROVIRAL THERAPY ON METABOLIC SYNDROME

In this chapter, I present a meta-analysis of the association between antiretroviral therapy and metabolic syndrome. I also identify study-level factors that may influence the pooled estimate.

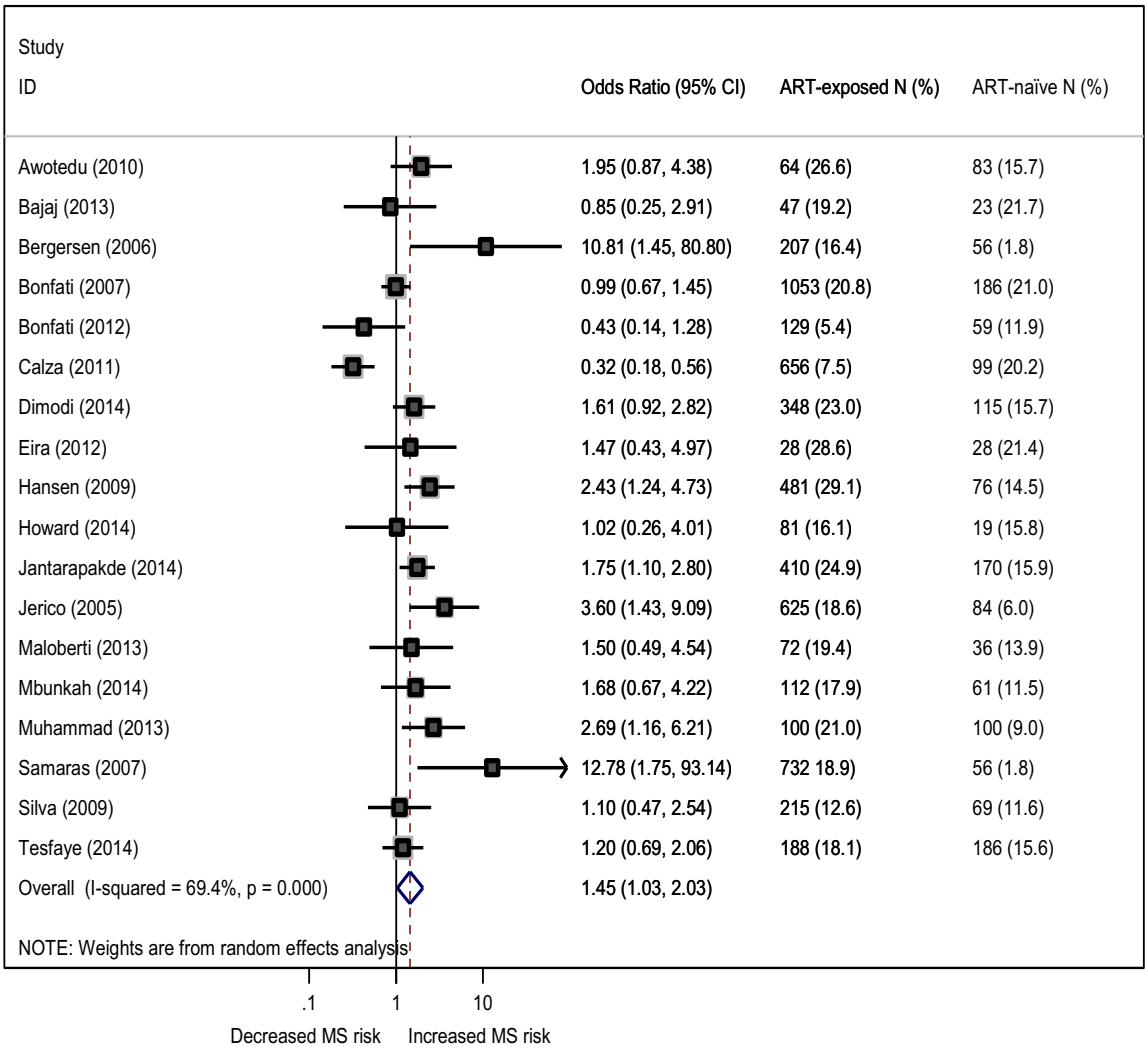
11.1 OVERALL ASSOCIATION BETWEEN ANTIRETROVIRAL THERAPY AND METABOLIC SYNDROME

11.1.1 Increase in the risk of metabolic syndrome

Prevalence estimates of metabolic syndrome were compared between antiretroviral-exposed and antiretroviral-naïve patients in 18 studies, 14 of which reported higher estimates among antiretroviral-exposed patients (Awotedu *et al*., 2010; Bergersen *et al*., 2006; Dimodi *et al*., 2014; Eira *et al*., 2012; Hansen *et al*., 2009; Howard *et al*., 2014; Jantarapakde *et al*., 2014; Jerico *et al*., 2005; Maloberti *et al*., 2013; Mbunkah *et al*., 2014; Muhammad *et al*., 2013; Samaras *et al*., 2007; Silva *et al*., 2009; Tesfaye *et al*., 2014) (Figure 11.1). Among 5,548 antiretroviral-exposed patients with reported metabolic syndrome status, 1048 (18.9%) were diagnosed with metabolic syndrome, compared to 214 (14.2%) of 1,506 patients in the antiretroviral-naïve group. Overall, the odds ratios of metabolic syndrome were significantly higher among antiretroviral-exposed patients, compared to their antiretroviral-naïve counterparts (Pooled OR 1.45, 95% CI 1.03 to 2.03). Heterogeneity across the included studies was substantial (I^2 = 69.4%) and statistically significant ($P < 0.001$). Funnel plot asymmetry was absent, suggesting no evidence of publication bias ($P = 0.175$ for Egger's regression test for funnel plot

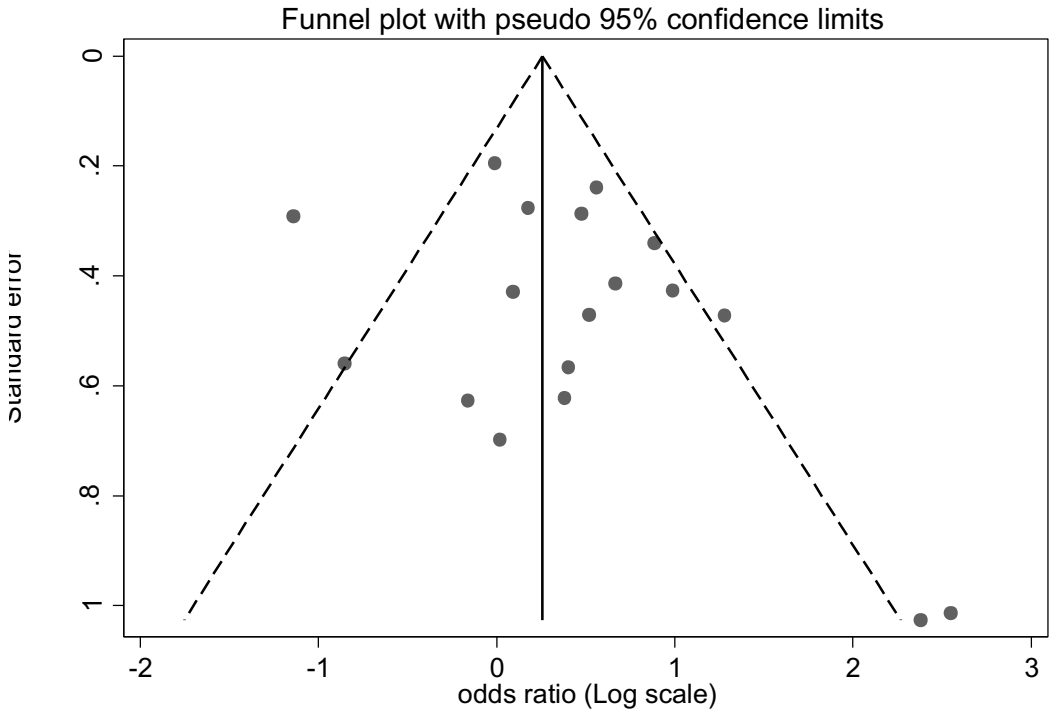
asymmetry) (Figure 11.2). Sensitivity analysis revealed no significant change in the pooled estimate following sequential omission of the included studies (Table 11.1).

Figure 11.1: Meta-analysis of the association between antiretroviral therapy and metabolic syndrome



ART, antiretroviral therapy; CI, confidence interval; MS, metabolic syndrome; N, total number of participants in each group; OR, odds ratio. The grey boxes and horizontal lines represent the odds ratios of metabolic syndrome with 95% CI in each study. The interrupted vertical line through the diamond represents the pooled odds ratio, whereas the two lateral vertices of the diamond represent the 95% CI of the pooled odds ratio. The solid vertical line through 1 represents no effect.

Figure 11.2: Analysis of publication bias in the association between antiretroviral therapy and metabolic syndrome



Funnel plot showing odds ratios of metabolic syndrome for each of the nine included studies plotted against the standard errors of the log odds ratio. The eighteen grey circles correspond to the eighteen studies included in the meta-analysis. *P* = 0.175 for Egger's test for small-study effects.

Table 11.1: Sensitivity analysis of the association between antiretroviral therapy and metabolic syndrome

Cardio-metabolic outcomes	Pooled OR (95% CI) prior to serial exclusion of individual studies	Range of pooled OR following serial exclusion of individual studies	Instance in which exclusion of a study significantly changed the pooled OR
Metabolic syndrome	1.45 (1.03 to 2.03)	1.42 to 1.59	None

CI, confidence interval; OR, odds ratio.

11.2 FACTORS THAT MAY INFLUENCE THE EFFECT OF ANTIRETROVIRAL THERAPY ON METABOLIC SYNDROME

Subgroup and meta-regression analyses revealed no significant differences between subgroup estimates of the pooled association between antiretroviral therapy and metabolic syndrome (Table 11.2). Nonetheless, the analyses revealed qualitative differences in the pooled effect between study-level subgroups of age, sex and country income group. For instance, antiretroviral therapy was significantly associated increased risk of metabolic syndrome among patients who were 40 years of age and older (Pooled OR 2.34, 95% CI 1.22 to 4.49), but not among patients who were younger (Pooled OR 1.14, 95% CI 0.67 to 1.95). Studies with more women than men reported a significant pooled association of antiretroviral therapy with increased risk of metabolic syndrome (Pooled OR 1.66, 95% CI 1.28 to 2.14), whereas no significant association was observed in studies with fewer women than men (Pooled OR 1.49, 95% CI 0.81 to 2.72). Antiretroviral therapy was also associated with increased risk of metabolic syndrome among HIV-infected patients resident in low- and middle-income countries (Pooled OR 1.61, 95% CI 1.26 to 2.04), but not among those resident in high-income settings (Pooled OR 1.27, 95% CI 0.63 to 2.53).

Table 11.2: Subgroup estimates and meta-regression analyses of the association between antiretroviral therapy and metabolic syndrome

			Meta-regression	
Study-level characteristics	**N**	**Pooled OR (95% CI)**	***P*-value**	**R^2 (%)**
Region				
Sub-Saharan Africa	5	1.62 (1.19 to 2.20)		
Europe	8	1.27 (0.63 to 2.53)		
South-East Asia	1	0.85 (0.25 to 2.91)		
The Americas	2	1.20 (0.60 to 2.40)		
Western Pacific	1	1.75 (1.10 to 2.80)		
Multi-regional	1	12.78 (1.75 to 93.14)	0.818	0
Income group				
High income	8	1.27 (0.63 to 2.53)		
Low/middle	10	1.61 (1.26 to 2.04)	0.841	0
Females				
< 50%	7	1.49 (0.81 to 2.72)		
≥ 50%	6	1.66 (1.28 to 2.14)	0.701	0
Age group				
< 40 years	8	1.14 (0.67 to 1.95)		
≥ 40 years	7	2.34 (1.22 to 4.49)	0.845	0
Study design				
Case-control	1	12.78 (1.75 to 93.14)		
Cohort	1	0.43 (0.14 to 1.08)		
Cross-sectional	16	1.45 (1.04 to 2.02)	0.610	3.1
Selection bias				
High/unclear risk	15	1.41 (0.97 to 2.06)		
Low risk	3	1.94 (0.54 to 6.97)	0.769	0

N, number of studies; OR, odds ratio; R^2, explained variability. Smoking status, HIV duration, antiretroviral regimen and antiretroviral treatment duration were excluded because data on these characteristics were insufficient to perform meta-regression analyses.

PART C

AN EPIDEMIOLOGICAL EXAMINATION OF THE ASSOCIATION BETWEEN ANTIRETROVIRAL THERAPY AND INCREASED BLOOD PRESSURE USING HILL'S CRITERIA OF CAUSATION (MINI-REVIEW)

CHAPTER TWELVE

BACKGROUND

In this chapter, I briefly set the stage for examining the extent to which the epidemiological association of antiretroviral therapy with increased blood pressure fulfills Hill's criteria of causation.

12.1 BACKGROUND

Although blood pressure changes have a multifactorial aetiology among people living with HIV, greater emphasis seems to be placed on the role of antiretroviral therapy, as opposed to other risk factors (Dau & Holodniy, 2008). In non-communicable disease epidemiology, the transition from association to causation cannot be overemphasized, and is seen as a necessary step for implementing preventive interventions at the population level (Lucas & McMichael, 2005). However, this transition is yet to come about with regards to the association between antiretroviral therapy and high blood pressure.

For more than half a century, the "Bradford Hill Criteria" have provided the background framework for assessing the causal nature of epidemiological associations (Hill, 1965). Basically, the evidence base for concluding that there is a high probability that a cause and an effect exists in an observed epidemiological association is determined by these criteria, which are outlined in Box 12.1 below. Therefore, it would be remiss of the author not to detail these criteria and how they may be applied to the association of antiretroviral therapy with increased blood pressure in people living with HIV.

Box 12.1: Hill's criteria of causation and their definitions

Criteria	Definitions
1. Temporal relationship	The exposure always precedes the outcome.
2. Strength	The size of the association as measured by appropriate statistical tests.
3. Dose-response relationship	An increasing measure of the exposure (in amount or time) increases the outcome measure
4. Consistency	The association between the exposure and the outcome is replicated across different studies, settings, and times.
5. Biological plausibility	The association between the exposure and the outcome is compatible with currently accepted understanding of the pathological mechanisms.
6. Analogy	The extent to which alternate explanations of the association between a characteristically similar exposure and the outcome have been ruled out.
7. Experiment	Evidence that the outcome can be modified or prevented using an appropriate experimental regimen.
8. Specificity	Besides the outcome in question, the exposure produces no other outcome. Similarly, the aetiology outcome is not multifactorial.
9. Coherence	The association between the exposure and the outcome is consistent with existing theory and knowledge.

Adapted from Hill (1965) and Mirtz *et al*. (2009).

CHAPTER THIRTEEN

METHODS

In this chapter, I describe the search strategy for identifying studies potentially relevant to the purpose of this mini-review. I also explain how each criterion of causation was to be assessed with respect to the association of antiretroviral therapy with increased blood pressure.

13.1 METHODS

I searched the databases of Embase, MEDLINE (OVID) and SciELO for studies considered eligible for inclusion in this review. The searches were limited to studies published between January 1997 and March 2016, and were conducted using the following MeSH terms and keywords: *highly active antiretroviral therapy/, antiretroviral therapy.mp./, *blood pressure/, *systolic blood pressure/, *diastolic blood pressure/, high blood pressure.mp/, *hypertension/, temporality.mp./, temporal relationship.mp./, odds ratio.mp./, *risk/, relative risk.mp./, *effect size/, biological plausibility.mp./, coherence.mp./, and analogy.mp./. The MeSH terms — denoted by the prefix '*' — were exploded to yield more specific terms; all the search terms were combined using Boolean operators. Additionally, data from the main findings of the meta-analyses of the associations between antiretroviral therapy and blood pressure/hypertension reported in the previous chapter were identified as pertinent to the purpose of the present review. To reiterate, the meta-analyses in question represent the most comprehensive evidence yet regarding the impact of antiretroviral therapy on blood pressure changes (and hypertension risk), providing the best possible source of information for the present review.

Each criterion of causation (Hill, 1965) was examined as follows:

I. *Temporal relationship*: This criterion was fulfilled if antiretroviral therapy always preceded increases in blood pressure, and never the other way around. Hill (1965) considered the criterion absolutely essential for establishing a causal inference. In other words, the absence of this criterion detracted much from the cause-effect relationship between antiretroviral therapy and increased blood pressure.

II. *Strength*: This criterion was fulfilled if the mean blood pressure or prevalence of hypertension was many times greater among antiretroviral-exposed patients, compared to their naïve counterparts. The larger the mean difference in blood pressure or the risk of hypertension, the more likely that exposure to antiretroviral therapy caused high blood pressure.

III. *Dose-response relationship*: This criterion was fulfilled if there was a direct association between increasing durations of antiretroviral therapy and increasing levels of blood pressure or prevalence estimates of hypertension. Conversely, the absence of this association detracted from the causal nature of antiretroviral therapy and increased blood pressure.

IV. *Consistency*: This criterion was fulfilled if the associations of antiretroviral therapy with increasing blood pressure and hypertension risk were replicated across many studies of different designs, and in different geographic or income settings, and at different times.

V. *Biologic plausibility*: This criterion was fulfilled if the association of antiretroviral therapy with increased blood pressure was coherent with current knowledge of the pathogenic mechanisms.

VI. *Analogy / consideration of alternate explanations*: This criterion was fulfilled if hypotheses of the hypertensive effects of other pharmacologic agents besides

antiretroviral therapy — preferably prescription drugs — or other potential factors with hypertensive effects were ruled out in HIV-infected patients.

VII. *Experiment*: This criterion was fulfilled if there was experimental evidence alluding to the prevention or reversal of increased blood pressure in HIV-infected patients (or animal models) following modification of antiretroviral therapy. Of note, modifications to antiretroviral treatment would not entail the complete withdrawal of this treatment in human subjects, given that HIV-infected patients eligible to commence antiretroviral therapy would succumb to AIDS without treatment.

VIII. *Specificity*: This criterion was fulfilled if there was a one-to-one cause-effect relationship between antiretroviral therapy and increased blood pressure. In other words, no other factor, besides antiretroviral therapy, was associated with increased blood pressure among people living with HIV. However, this criterion is considered to be the weakest of all the criteria, in the context of chronic multifactorial conditions such as high blood pressure, so that its absence does not detract from the cause-effect relationship between antiretroviral therapy and increased blood pressure.

IX. *Coherence*: This criterion was fulfilled if the association between antiretroviral therapy and increased blood pressure was consistent with existing knowledge within the field.

CHAPTER FOURTEEN

RESULTS

In this chapter, I report the results of the epidemiological examination of the association between antiretroviral therapy and increased blood pressure based on each of Hill's criteria of causation.

14.1 RESULTS

I. Hill's criteria and the temporal relationship between antiretroviral therapy and increased blood pressure.

Antiretroviral therapy always precedes increases in blood pressure; it is theoretically impossible that the reverse could ever be the case. Although the literature search did not yield any temporal sequence studies, the Multicentre AIDS Cohort Study, which is the largest ongoing prospective study designed to examine the natural history of HIV infection and AIDS, reported a temporal relationship between the exposure to antiretroviral therapy and increases in the incidence of systolic and diastolic hypertension (Seaberg *et al*., 2005).

II. Hill's criteria and the strength of association between antiretroviral therapy and increased blood pressure.

Antiretroviral-exposed HIV-infected patients may be up to ten times more likely to be hypertensive, compared to their naïve counterparts (OR 10.04, 95% CI 2.25 to 44.71) (Ekali *et al*., 2013). The mean systolic (MD 12.72 mmHg, 95% CI 9.10 to 16.34) and diastolic blood pressure (MD 7.57 mmHg, 95% CI 5.00 to 10.14) have been reported to

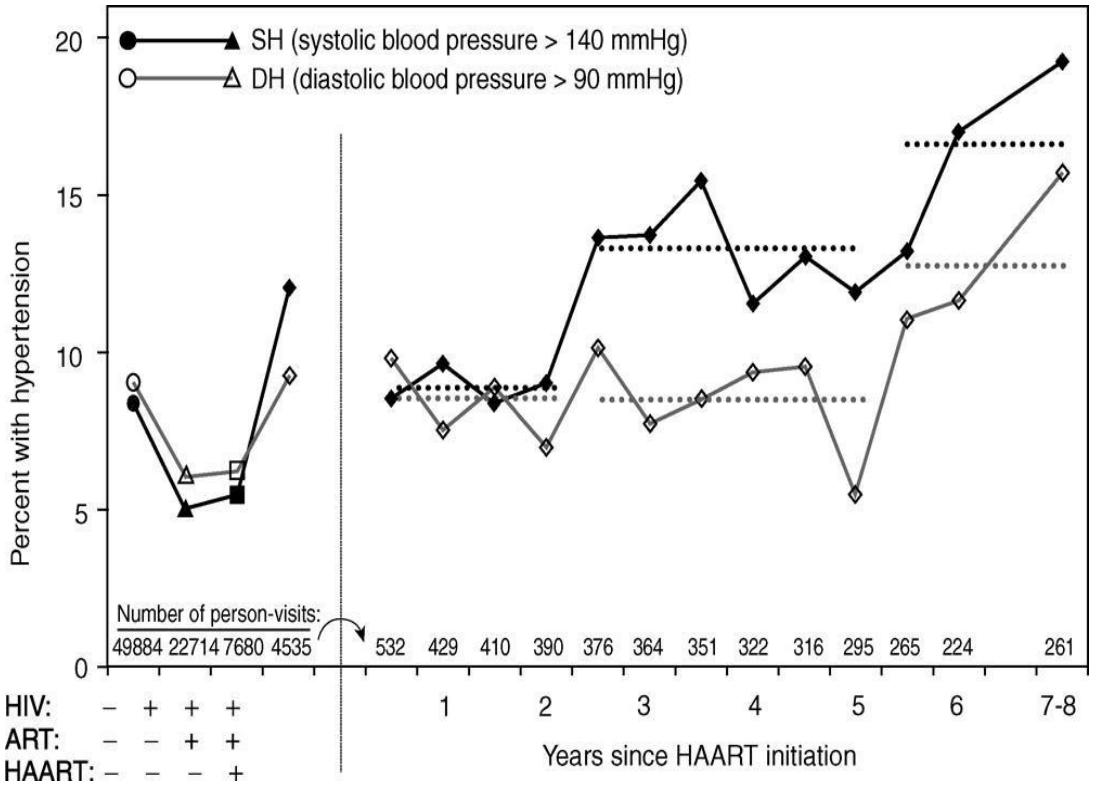
be substantially greater in antiretroviral-exposed patients than antiretroviral-naïve patients by more than 12 mmHg and 7 mmHg respectively (Mital *et al*., 2013). More precisely, the mean systolic (MD 4.52 mmHg, 95% CI 2.65 to 6.39) and diastolic blood pressure (MD 3.17 mmHg, 95% CI 1.71 to 4.64) were greater in antiretroviral-exposed patients, compared to their naïve counterparts, by more than 4mmHg and 3 mmHg respectively, after pooling the effect estimates from different studies.

III. Hill's criteria and the dose-response relationship between antiretroviral therapy and increased blood pressure.

Data from the MACS study revealed increases in the incidence of systolic and diastolic hypertension as the duration of antiretroviral therapy increased (Seabgerg *et al*., 2005). Figure 14.1 below is a graphical illustration of the prevalence of systolic and diastolic hypertension by duration of antiretroviral therapy as depicted in the MACS study (Seaberg *et al*., 2005). Furthermore, subgroup and meta-regression analyses performed in the evidence synthesis to examine the impact of antiretroviral treatment duration on the pooled association between antiretroviral therapy and hypertension risk revealed a significantly higher risk of hypertension in HIV-infected patients on antiretroviral therapy for more than 18 months (pooled OR 2.23, 95% CI 1.55 to 3.20); whereas no significant association was observed on antiretroviral therapy for less than 18 months (pooled OR 1.81, 95% CI 0.65 to 5.04)

(see Table 7.4).

Figure 14.1: Prevalence of systolic hypertension (SH) and diastolic hypertension (DH) by HIV sero-status and therapy, and relative to the time of highly active antiretroviral therapy (HAART) initiation



The dotted lines represent the mean prevalence of SH and DH for the three categories of the duration of HAART exposure included in the multiple regression analyses: less than 2 years, 2 to 5 years, and more than 5 years. ART, antiretroviral therapy.
Source: Multicentre AIDS Cohort Study (Seaberg *et al*., 2005).

IV. Hill's criteria and consistency in the association between antiretroviral therapy and increased blood pressure.

The pooled associations of antiretroviral therapy with increased systolic (pooled MD 4.52 mmHg, 95% CI 2.65 to 3.69) and diastolic blood pressure (pooled MD 3.17 mmHg, 95% CI 1.71 to 4.64) and hypertension risk (pooled OR 1.68, 95% CI 1.35 to 2.10) were statistically significant across different studies (see meta-analyses in Figures 7.1, 7.3 & 7.5). In addition, the pooled associations in the evidence synthesis were statistically significant across study

designs, geographical regions, country income groups, and publication years (see results of subgroup and meta-regression analyses in Tables 7.2 to 7.4).

V. Hill's criteria and the biological plausibility of the association between antiretroviral therapy and increased blood pressure.

The pathologic basis of antiretroviral-associated increases in blood pressure has been linked to direct damage to the endothelial linings of blood vessels by antiretroviral drugs, which subsequently interferes with the production of biological markers known to regulate blood pressure — notably nitric oxide (Ngatchou *et al*., 2013; Seaberg *et al*., 2005; Stein, 2003). Rising blood pressure levels may also be symptomatic of an exaggerated inflammatory response following the initiation of antiretroviral therapy in the advanced stages of HIV infection (Bosamiya, 2011).

VI. Hill's criteria and the consideration of alternate hypotheses of the association between antiretroviral therapy and increased blood pressure.

The iatrogenic causes of hypertension in the general population have been well documented. For instance, one in five patients treated with synthetic steroids presents with hypertension (Grossman & Messerli, 2004). Given the increased potential for steroidal abuse, this proportion may be underestimated. In high-risk groups, the prevalence of prescription drug abuse tends to be higher in comparison with the general population. For instance, prescription drugs have been identified as the most common drugs of abuse among people living with HIV, accounting for a pooled prevalence estimate of 42.7% (Nduka *et al*., 2015a). However, the literature search yielded no studies that offered a consistent analogy linking high blood pressure with other prescription drugs such as corticosteroids or anabolic steroids in HIV-infected patients.

VII. Hill's criteria and the experimental evidence for the association between antiretroviral therapy and increased blood pressure.

In a clinical trial aimed to examine the effectiveness of switching antiretroviral regimens on lipids and cardiovascular risk in HIV-infected patients, Rokx *et al*. (2015) found that switching from a first generation to a second generation non-nucleoside reverse transcriptase inhibitor-based treatment produced a statistically significant decrease in systolic blood pressure (MD -6 mmHg, 95% CI -1.7 to -10.3; *P* = 0.007). However, this was a single small study comprising just 50 participants, and given the wide confidence intervals of the effect estimate, it is not clear how replicable this finding is. In addition, evidence that is obtained from small trials tend to be susceptible to publication and selective reporting biases (Loannidis, Cappelleri & Lau, 1998), and small trials may erroneously show larger treatment effects (Pereira, Horwitz & Loannidis, 2012). No experimental animal studies were found that examined the effects of complete withdrawal of antiretroviral therapy on blood pressure changes.

VIII. Hill's criteria and the specificity of the association between antiretroviral therapy and increased blood pressure.

The association of antiretroviral therapy with increased blood pressure does not satisfy Hill's criterion of specificity. For instance, antiretroviral therapy has been associated with several other cardio-metabolic conditions besides increased blood pressure, such as: dyslipidaemia, overweight/obesity, diabetes mellitus, and metabolic syndrome (Dau & Holodniy, 2008; Dagogo-Jack, 2008; Troll, 2011). Similarly, the aetiology of high blood pressure, especially among people living with HIV, is multifactorial. For instance, HIV- infected patients are more likely to smoke, drink hazardously, and consume larger quantities of dietary fat, compared to HIV-negative individuals (Jaime *et al*., 2006; Joy *et al*., 2007; Saves *et al*., 2003). Nonetheless, it is worth noting that

these traditional risk factors of hypertension may be limited in predicting blood pressure changes among people living with HIV on antiretroviral therapy (Troll, 2011). Moreover, this criterion of causation is considered the weakest of all the criteria, especially in the context of chronic multifactorial conditions such as high blood pressure, so that its absence does not detract from the cause-effect relationship between antiretroviral therapy and increased blood pressure (Hill, 1965).

IX. Hill's criteria and coherence in the association between antiretroviral therapy and increased blood pressure.

In addition to antiretroviral-induced endothelial dysfunction, the association between antiretroviral therapy and increased blood pressure is coherent with the temporal rise in the latter that has taken place since the advent of HAART two decades ago, among HIV-infected people (Hooshyar *et al*., 2007; Seaberg *et al*., 2005).

PART D

PRIMARY DATA COLLECTION AND ANALYSIS

IN A RESOURCE-LIMITED SETTING

I have published parts of this section in the following journals:

I. **Chidozie U Nduka**, Olalekan A Uthman, Peter K Kimani, Abraham O Malu, Saverio Stranges. Body fat changes mediate the effects of antiretroviral therapy on blood pressure and blood glucose levels in people living with HIV in a sub-Saharan African setting: a mediation analysis (Abstract). *Circulation* 2016; 133: AP079 (Abstract).

II. **Chidozie U Nduka**, Olalekan A Uthman, Peter K Kimani, Abraham O Malu, Saverio Stranges. Impact of body fat changes in mediating the effects of antiretroviral therapy on blood pressure in HIV-infected persons in a sub-Saharan African setting. *Infectious Diseases of Poverty* 2016; 5(1); DOI: 10.1186/s40249-016-0152-7.

III. **Chidozie U Nduka,** Saverio Stranges, Gerald S Bloomfield, Peter K Kimani, Godwin Achinge, Abraham O Malu, Olalekan A Uthman. A plausible causal link between antiretroviral therapy and increased blood pressure in a sub-Saharan African setting: a propensity score-matched analysis. *International Journal of Cardiology* 2016; 220: 400 – 407.

CHAPTER FIFTEEN

BACKGROUND, AIMS AND OBJECTIVES

In this chapter, I describe the scientific context for investigating a plausible causal link between antiretroviral therapy and increased blood pressure using appropriate statistical methods. I also outline the specific objectives of this section.

15.1 BACKGROUND

The nature of the epidemiological association between antiretroviral therapy and increased blood pressure is complex and has not been fully elucidated. For instance, it remains largely unknown whether the association between antiretroviral therapy and increased blood pressure is causal in nature. A plausible reason for this trend might be that the available studies on the subject tend to be observational. It is widely acknowledged that randomised controlled trials provide a higher level of evidence suggestive of causality, compared to observational studies. However, what happens when it becomes impractical to answer certain research questions using controlled experiments? Such questions have led to a growing interest in using observational data to infer or rule out causal effects (Austin, 2011a; Austin, 2011b; Kenny, 2015; Thenappan *et al*., 2014). Nonetheless, technically, it is not so much a growing interest than it is a renewed interest, given that the primary objective of observational studies has always been to infer causality in those situations where controlled experimentation proves unrealistic (Cochran, 1965). The association between antiretroviral therapy and increased blood pressure falls within this category, given that the random assignment of HIV-infected subjects — who might be eligible to commence antiretroviral treatment — to a control group, where they are administered placebos would inevitably raise significant ethical concerns.

Furthermore, as modern-day epidemiology continues to evolve, the interpretations of causality continue to expand, above and beyond the simple direct relationships that fulfil Hill's criteria of causation, to account for the multifactorial aetiology of most chronic diseases (Lucas & McMichael, 2005; Mirtz *et al*., 2009). Statistical methods have become increasingly fundamental to this purpose by identifying different potential causal pathways that reflect the complex interplay of risk factors that explain the pathogenic processes associated with most chronic diseases nowadays (Henneken & Buring, 1987; Kenny, 2015; Lucas & McMichael, 2005; Mirtz *et al*., 2009). However, these statistical concepts have not been applied to the epidemiological association between antiretroviral therapy and increased blood pressure. Unfortunately, statistical methods in previous studies that have examined this association have been limited to standard regression techniques, which do not elucidate the potential mechanisms through which blood pressure changes may occur following antiretroviral therapy.

Of note, it was essential to address this gap in the current body of evidence, as the findings could be useful in uncovering the complex epidemiology of hypertension and other cardio-metabolic disorders among people living with HIV in the sub-Saharan African region, which in turn, may guide preventive action.

15.2 AIMS AND OBJECTIVES

There are three aims in this section.

15.2.1 The first aim

To examine the impact of antiretroviral treatment status on the clinical prediction of hypertension in people living with HIV in a sub-Saharan African setting.

15.2.1.1 Objectives

I. To develop the best parsimonious model for predicting hypertension using traditional (and non-traditional) risk factors in people living with HIV in a sub-Saharan African setting.

II. To examine whether or not the addition of antiretroviral treatment status to the predictive model improves the clinical prediction of hypertension in people living with HIV in a sub-Saharan African setting.

15.2.2 The second aim

To examine alternative causal pathways that potentially mediate the effects of antiretroviral therapy on blood pressure in people living with HIV in a sub-Saharan African setting.

15.2.2.1 Objectives

I. To examine the indirect effects of antiretroviral therapy on blood pressure through changes in body fat measures in people living with HIV in a sub-Saharan African setting.

II. To examine the indirect effects of antiretroviral therapy on blood pressure through changes in blood glucose concentration in people living with HIV in a sub-Saharan African setting.

III. To examine the indirect effects of antiretroviral therapy on blood pressure through changes in sleep quality in people living with HIV in a sub-Saharan African setting.

15.2.3 The third aim

To estimate the average treatment effect on the treated (ATT) of antiretroviral therapy on blood pressure in people living with HIV in a sub-Saharan African setting.

15.2.3.1 Objectives

I. To match the characteristics of HIV-infected patients naïve and exposed to antiretroviral therapy based on estimated propensity scores.

II. To estimate the ATT of antiretroviral therapy on blood pressure in the propensity score-matched sample.

CHAPTER SIXTEEN

METHODS

In this chapter, I specify the methods for primary data collection and analysis. I also describe the rationales for these methods.

16.1 STUDY DESIGN

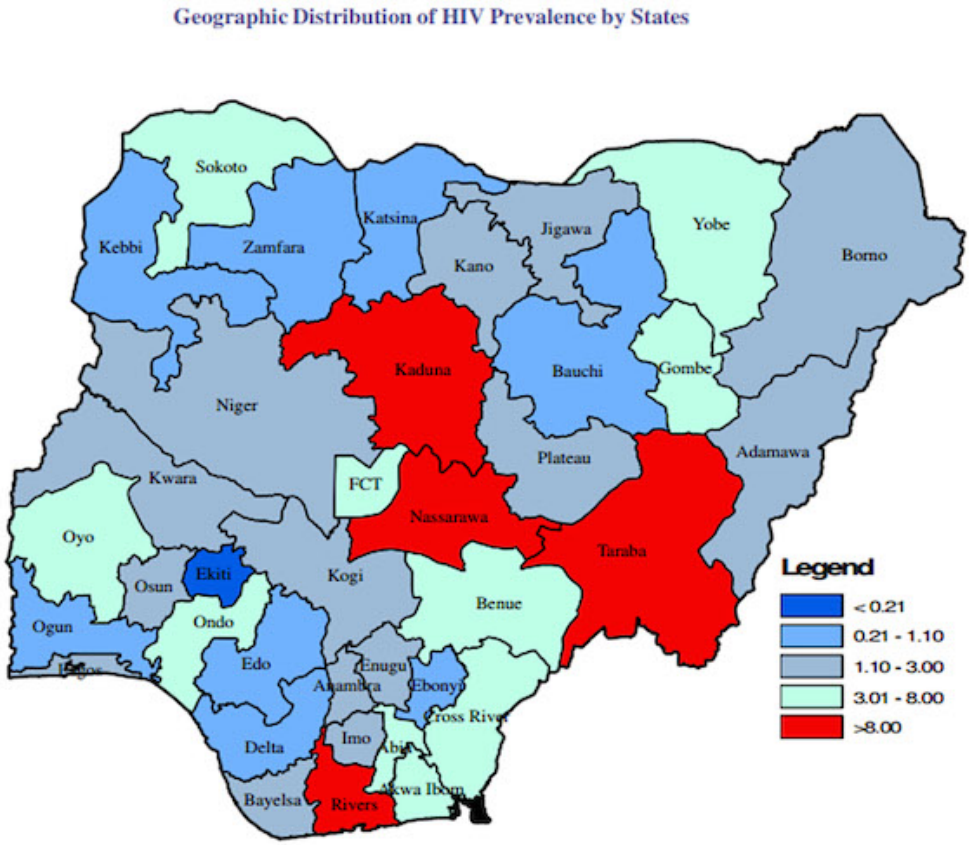
A cross-sectional design was deemed appropriate to achieve the study aims and objectives. A prospective cohort study may provide the highest level of evidence among observational studies, especially by eliminating the potential for reverse causality since the design inherently entails a temporal relationship between antiretroviral therapy and blood pressure changes. However, blood pressure changes do not lead to antiretroviral exposure, so that reverse causality in the association between antiretroviral therapy and blood pressure changes is biologically impossible, and was unlikely to invalidate the findings of the present study. Furthermore, the cross-sectional design was a pragmatic choice, given that it is relatively inexpensive and more compatible with the timeframe of a doctoral programme.

16.2 STUDY SETTING

Consenting HIV-infected patients attending the HIV clinic at the Benue State University Teaching Hospital in Makurdi, Nigeria were recruited between August and November 2014, as part of a cross-sectional study. During this period, over 700,000 people were reported to be living with HIV in Benue state, accounting for 5.6% of the resident population in the state, and is higher than the current national average of 3.6% (National Agency for the Control of AIDS, 2014). The Benue State University Teaching Hospital is one of two tertiary health institutions providing HIV/AIDS care and treatment services

for the resident population of Benue. Approximately 50 patients visit the HIV clinic on a typical day and enrolment into care usually coincides with the date HIV-infection was first diagnosed. Until two years ago, Benue state had consistently recorded the highest prevalence of HIV across all states in Nigeria, accounting for more than 10% of its resident population throughout the preceding decade (National Agency for the Control of AIDS, 2012). Between 2003 and 2010, HIV prevalence in Benue state increased by more than 2% (10.5% to 12.7%), with all-cause mortality more than doubling (1.1 to 2.4 per 100,000 people); whereas, the national prevalence of HIV decreased by 0.9% (5.0% to 4.1%) during the same period (National Agency for the Control of AIDS, 2012). Figure 16.1 is an image showing the most recent mapping of HIV prevalence by state in Nigeria.

Figure 16.1: Map of Nigeria showing HIV prevalence by state (2014)



Source: National Agency for the Control of AIDS, 2014.

Located in the Mid-East region of Nigeria, Benue state is endowed with more varieties of agricultural products than any other state in Nigeria (National Bureau of Statistics, 2012). However, the food supply in Benue state has not translated into higher living standards for the resident population. In 2007, the Gross Domestic Product (GDP) Income Per Capita of the state was $1,592 (National Bureau of Statistics, 2012), which is low by World Bank (2015) standards. Such high levels of socio-economic deprivation in Benue contribute substantially to the HIV epidemic in the state (Utulu & Lawoyin, 2007).

The Benue State University Teaching Hospital is located in the capital city of Makurdi, which is home to approximately 10% of the five million people domiciled in the state (National Bureau of Statistics, 2012). Makurdi is located close to other cities with high HIV prevalence such as Abuja (8.6%), Calabar (7.1%) and Jos (7.7%) (National Agency for the Control of AIDS, 2014), and there are high volumes of traffic between these cities, mainly for economic reasons. In other words, the high prevalence of HIV in Benue state may also be partially attributed to the close proximity and interaction of Makurdi with these cities (Djukpen, 2012). Furthermore, certain "socio-cultural practices such as wife/widow inheritance, spouse sharing, female circumcision and polygamy" are quite prevalent in Makurdi as they are in its neighbouring cities, further propagating the HIV disease epidemic in Benue state (Djukpen, 2012; p. 118).

Importantly, patients attending the HIV clinic at the Benue state University Teaching Hospital are somewhat comparable to those attending other HIV clinics in the state and across the country: HIV infection is as much an infectious disease of poverty in Benue state as it is in other states in Nigeria, as well as in other countries in sub-Saharan Africa.

16.3 CRITERIA FOR SELECTING STUDY PARTICIPANTS

16.3.1 Inclusion criteria

I. HIV-infected patients

II. 18 years old and above

III. Naïve or exposed to highly active antiretroviral therapy (HAART). However, patients exposed to HAART had to be on antiretroviral treatment for at least three months

IV. Nigerian pidgin or English speaking

V. HIV-infected patients in clinical stages I and II as classified by the World Health Organization (2005). Patients in HIV clinical stage I are usually asymptomatic, but may present with persistent generalized lymph nodes, with predilection for the axillary, cervical and inguinal regions of the body. Patients in HIV clinical stage II present with one or more of the following relatively mild illnesses: moderate unexplained weight loss that is less than 10% of presumed or measured body weight; recurrent respiratory tract infections (such as sinusitis, bronchitis, otitis media, pharyngitis); herpes zoster infection; angular cheilitis (an inflammatory condition affecting the corners of the mouth); recurrent oral ulcerations; papular pruritic eruptions; seborrhoeic dermatitis (a condition where the skin appears erythematous, inflamed and itchy); and fungal infections affecting the nails of the fingers (World Health Organisation 2005).

16.3.2 Exclusion criteria

I. Adolescents and children

II. Sub-optimal adherence to regular follow-up visits at the HIV clinic. Antiretroviral adherence levels in such patients were likely to be suboptimal, which may have precluded the essence of antiretroviral exposure, given that antiretroviral medications were only re-filled during follow-up visits. Adherence levels to follow-up visits at the clinic were

determined from patients' medical records, and were considered inadequate if the last two follow-up visits — usually one month apart — prior to the study were missed consecutively. It is worth emphasizing that this definition is somewhat arbitrary, given that there is no universally accepted definition for antiretroviral adherence (University of California, San Francisco [UCSF] Centre for HIV Information, 2016).

III. Pregnant and lactating mothers: as part of the prevention of mother-to-child transmission of HIV (PMTCT) guidelines, all pregnant and breastfeeding mothers must be administered antiretroviral prophylaxis (World Health Organisation, 2010a).

IV. HIV-infected patients for whom their most recent CD4 cell count assessments were carried out more than three months prior to commencing the study. According to the HIV/AIDS care and treatment guidelines in Nigeria, HIV-infected patients are required to undergo CD4 count testing every three months (Federal Ministry of Health Nigeria, 2012).

V. Patients presenting with persistent decline in CD4 cell counts or with clinically symptomatic HIV infection while on antiretroviral therapy for no less than three months. Such patients, upon identification, were diagnosed of antiretroviral treatment failure (AIDS info, 2015).

VI. Patients presenting in any of the more advanced clinical stages of HIV/AIDS often characterized by the presence of opportunistic infections: World Health Organisation clinical stage III and stage IV. Such patients were deemed ineligible for the study *ab initio* because they were more likely to be in pain and clinically unstable (subnormal or elevated body temperatures, pulse rates, respiratory rates and blood pressure readings), compared to patients in the less advanced clinical stages I and II. Generally, patients in WHO clinical stage III of HIV/AIDS present with one or more of the following signs and symptoms: severe weight loss that is greater than 10% of presumed or measured body weight;

unexplained chronic diarrhoea that has persisted for more than one month; unexplained intermittent or constant fever that has persisted for longer than one month; oral candidiasis (a fungal infection caused by *Candida species* and present in the buccal mucosa); oral hairy leukoplakia (an oral lesion caused by the Epstein-Barr virus that presents as a white, often hairy-looking, patch on the tongue); a history of pulmonary tuberculosis (TB) diagnosed within the last two years; necrotizing periodontal diseases such as acute necrotizing ulcerative stomatitis, gingivitis or periodontitis (severe bacterial infections affecting the teeth and gums); and severe bacterial infections affecting other organs and tissues in the body such as pneumonia (the lungs), empyema (a pus-filled cavity in any part of the body, but more commonly pleural in origin), pyomyositis (pus-filled abscesses in the skeletal muscles), bone or joint infection, meningitis (the meninges or membranes covering the brain and spinal cord), and bacteraemia (the blood); unexplained anaemia (a low red cell count in the blood), neutropenia (low white blood cell count) and thrombocytopaenia (low platelet count in the blood) for more than one month.

Patients in HIV clinical stage IV present with one or more of the following AIDS-defining illnesses: HIV wasting syndrome (patient loses more than 10% of their body weight accompanied by persistent diarrhoea, generalized weakness and fever for no less than one month); pneumocystis pneumonia (atypical lung infection caused by the fungus *Pneumocystis Jirovecii*); recurrent severe bacterial pneumonia; chronic herpes simplex infection affecting buccal, genital or anorectal areas for at least one month; oesophageal candidiasis (a fungal infection caused by the organism *Candida Albicans* and present in the oesophageal wall); tuberculosis affecting areas other than the lungs, notably the spine (tuberculosis of the spine) and the abdomen (intra-abdominal tuberculosis); Kaposi's sarcoma (a tumour of viral origins that affects the skin and internal organs); toxoplasmosis (caused by the parasite *Toxoplasma gondii*) affecting the central nervous system; HIV

encephalopathy (damage to the brain following chronic HIV infection); meningitis caused by *Cryptococcus species*; disseminated non-tuberculous mycobacteria infection; Progressive multifocal leukoencephalopathy (progressive inflammation of the white matter at different areas of the brain); tracheal, bronchial or pleural candidiasis; cryptosporidiosis (diarrheal disease caused by the parasite *Cryptosporidium species*); isosporiasis (disease affecting the intestines caused by the parasite *Isospora belli*); visceral herpes simplex infection; cytomegalovirus infection affecting the retina; any disseminated fungal infection; recurrent septicaemia following non-typhoidal salmonella infection; lymphoma (cerebral or B cell non-Hodgkin); invasive cervical carcinoma; and visceral leishmaniasis (the most severe form of leishmaniasis) (World Health Organisation, 2005).

16.4 SAMPLING STRATEGY

Participants who met the pre-determined criteria for inclusion were selected consecutively until a sample of 406 HIV-infected participants, comprising 306 antiretroviral-exposed and 100 antiretroviral-naive patients, was achieved. In addition to being less expensive and less time-consuming than probability sampling techniques that employ random selection, consecutive sampling was an appropriate strategy for the primary study, given that the criteria for selecting participants for the study were relatively stringent, potentially reducing the target population (Bowers, House & Owens, 2011). For instance, participants were eligible for the primary study on the basis of the following criteria: age, HIV infection status, antiretroviral treatment status, clinical staging of HIV infection, adherence levels to follow-up visits at the HIV clinic, pregnancy status (or lactation), most recent CD4 cell count investigation, clinical and immunological response to antiretroviral treatment, and language. The absence of an electronic database where the medical records

of all patients attending the HIV clinic are stored also limited access to a sampling frame from which to select the population sample randomly (Bowers, House & Owens, 2011). Moreover, previous evidence found no differential impact on antiretroviral treatment outcomes between HIV-infected participants recruited by random sampling and those recruited using consecutive sampling (Tassie *et al*., 2010).

A sample of 104 HIV-negative individuals who consented to participate in the study were also sampled consecutively (for descriptive purposes only) from patients attending the Benue State University Teaching Hospital medical outpatient clinic. HIV-negative individuals were mostly ambulatory patients attending their follow-up appointments at the outpatient clinic.

16.5 STUDY OUTCOMES

The outcomes of the study were systolic and diastolic blood pressure. Blood pressure was measured using the Omron M10 IT Blood Pressure Monitor, with the patient's back supported while in sitting position. The upper arm was supported at a level corresponding to the heart in order to avoid blood pressure readings that are either too high (in cases where the upper arm is supported below heart level) or too low (in cases where the upper arm is supported above heart level). Of note, blood pressure was measured on each patient's preferred upper arm. Where necessary (e.g. obese persons), the blood pressure cuff was replaced with an appropriate-sized cuff. The average of the first two blood pressure readings taken no less than 20 minutes apart was recorded as the patient's blood pressure (National Institute for Health and Care Excellence, 2011). A third blood pressure reading was taken in cases where the disparity between the first two systolic or diastolic blood pressure readings was 5 mmHg or greater, so that the lower of the second and third

measurements was recorded as the patient's blood pressure (National Institute for Health and Care Excellence, 2011). Hypertension was diagnosed as blood pressure of at least 140/90 mmHg, or the use of antihypertensive medication (National Institute for Health and Care Excellence, 2011).

16.6 EXPOSURE

The exposure criterion was highly active antiretroviral therapy (HAART) status. Exposure to HAART entailed treatment using two nucleoside reverse transcriptase inhibitors with either one non-nucleoside reverse transcriptase inhibitor (2NRTI + 1 NNRTI), or one protease inhibitor (2NRTI + 1PI), for at least three months. According to the HIV/AIDS care and treatment guidelines in Nigeria, Zidovudine/Lamivudine/Nevirapine (AZT/3TC/NVP), Zidovudine/Lamivudine/Efavirenz (AZT/3TC/EFV), Tenofovir/Lamivudine/Nevirapine (TDF/3TC/NVP) and Tenofovir/Lamivudine/Efavirenz (TDF/3TC/EFV) are the commonly prescribed first-line HAART regimens, whereas, the protease inhibitors, notably Ritonavir-boosted Lopinavir (LPV-r), are considered second-line (Federal Ministry of Health Nigeria, 2012). In contrast, a patient was considered HAART-naïve if they were HIV-infected but not eligible to commence antiretroviral treatment.

16.7 OTHER DATA SOURCES AND MEASUREMENTS

Other data obtained from each participant included demographic factors (such as age, gender, occupational grade, educational attainment); behavioural and lifestyle factors (such as smoking status, drinking status, physical activity, presence of depressive symptoms, and sleep quality); anthropometric indices (such as body mass index and waist circumference); random blood glucose concentration; baseline systolic and diastolic

blood pressure; co-morbidities (such as overweight/obesity and central obesity); HIV-related characteristics (such as CD4 cell count, duration of HIV infection, HAART status, and duration of HAART); and other factors including health-related quality of life and family history of hypertension. Overweight/obesity was defined as a body mass index of at least 25 kg/m^2, and central obesity was assessed as waist circumference of at least 80cm for women and at least 94cm for men (National Institute for Health and Clinical Excellence, 2011).

Socioeconomic data, including educational attainment and occupational grade, were collected using the stepwise approach to surveillance instrument (World Health Organisation, 2001). Educational attainment and occupation are considered as conventional indicators of socio-economic status as it pertains to health research (Duncan *et al*., 2002). Educational status was dichotomised based on the number of years of formal education completed. Typically, patients who spent more than 12 years of formal education (e.g. university undergraduates, graduates and postgraduates), versus patients who had completed no more than 12 years of formal education (e.g. the non-educated, and primary and secondary school leavers). Occupational grades were also dichotomised: participants who belonged to the middle class (e.g. clerks; low-level managers, administrators and professionals; intermediate-level managers, administrators and professionals; and high-level managers, administrators and professionals) and those below the middle class (e.g. the unemployed; pensioners; unskilled, semi-skilled and skilled manual workers) (Market Research Society, 2006). It is worth noting that none of the participants in this study belonged to the upper class (the wealthiest members of society who wield the greatest political powers), which was expected, given that such

individuals are not representative of the resident population in Nigeria (or in any other country in the world) (Akhbar-Williams, 2010; Glover, 2007).

All lifestyle data were self-reported, and were obtained using the World Health Organisation STEPS instrument (Appendix 2), which has been validated for chronic disease risk factor surveillance in low- and middle-income settings (World Health Organisation, 2001). Drinking status was classified as either 'current drinkers' or 'non-drinkers'. Among drinkers, alcohol intake was measured in units using the equation: *[Number of millilitres in alcoholic drink × Alcohol volume in %] / 1000* (Institute of Alcohol Studies, 2013). The local brew, palm wine, was considered because consumption levels are quite substantial. Palm wine is thought to have alcohol content of about 4%. Alcohol consumption categories were defined according to South African drinking guidelines as 21 units per week for men and 14 units per week for women (International Centre for Alcohol Policies, 2010). No such guideline exists for Nigeria (International Centre for Alcohol Policies, 2010). Smoking status was classified as either 'ever smokers' or 'never smokers'. According to the World Health Organisation (2010b) recommendations, physical activity levels were dichotomised as greater or less than 150 minutes weekly of moderate intensity physical activity. Essentially, the weekly amount of exercise was obtained from a combination of self-reports of the duration and frequency of physical activity in a typical week. Of note, physical activity was not restricted to regimented exercises, but included all energetic movements of the body in everyday life, such as house chores, manual jobs, mode of transportation, etc.

Capillary blood samples for all study participants were obtained to determine random glucose levels using the Accu-Check Aviva Nano blood glucose system, United Kingdom. The blood samples were obtained by two trained phlebotomists who were also members of the Benue State University Teaching Hospital staff.

Height (in meters) and weight (in kilograms) were measured using a stadiometer and a weighing scale respectively. The patient was required to stand in the anatomical position on a flat surface without footwear or hair covering. Body mass index was derived from both variables using the standard formula: weight (kg)/height^2 (m^2).

Sleep quality was assessed as 'good' or 'poor' using the Pittsburgh Sleep Quality Index (PSQI) (Appendix 3), which is a validated instrument comprising 19 self-rated questions designed to measure the quality and patterns of sleep in adults within the last one month (Buysse *et al*., 1989). The 19 self-rated questions assess sleep quality across seven domains including: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction (Buysse *et al*., 1989). The maximum obtainable PSQI score is 21; however, a patient with a score of 5 or more was considered to have poor sleep quality, while good sleep quality was defined as a PSQI score of less than 5 (Buysse *et al*., 1989).

All participants were screened for the presence of depressive symptoms using the Centre for Epidemiologic Studies Depression scale (CES-D) (Radloff, 1977). The CES-D scale is a 20-item questionnaire designed to measure self-rated symptoms associated with depression. Each item on the questionnaire is scored a value ranging from 0 to 3, where 0 indicates the absence or rare presence of the depressive symptom in question and 3

indicates presence of the symptom in question most or all of the time (Appendix 4). In other words, the obtainable CES-D scores range from 0 to 60 overall, with higher scores indicating greater depressive symptoms. The cut-off CES-D score below which a diagnosis of clinical depression is unlikely is 16, and this formed the basis for dichotomising the CES-D scores of participants in the present study (Radloff, 1977).

Health-related quality of life (HRQL) was assessed using the Medical Outcomes Study 12-item Short Form (SF-12) Survey, which has previously been validated on high-risk populations in sub-Saharan African settings (Bello-Mojeed *et al*., 2013). Participants responded to 12 questions designed to evaluate self-reported measurements of physical and mental well-being in eight areas: general health, physical functioning, role functioning as determined by physical factors, bodily pain, vitality, role functioning as determined by emotional factors, mental health, and social functioning (Ware, Kosinski & Keller, 1996). The Physical and Mental Health Composite Scores (PCS & MCS) derived from the 12 questions range from 0 to 100, with higher scores indicating better HRQL on the respective physical and mental health scales (Ware, Kosinski & Keller, 1996) (Appendix 5).

Baseline blood pressure referred to systolic and diastolic blood pressure of HIV-infected patients obtained at the time of enrolment into care (or HIV infection diagnosis). These data were obtained from the patients' medical records. It was important to adjust for these variables in order to determine whether the effects of antiretroviral therapy on blood pressure were influenced by blood pressure levels prior to HIV-infection diagnosis (or prior to the commencement of HAART). Other data including CD4 cell count, duration of HIV infection, HAART status, and duration of HAART were also obtained from the patients' medical records.

16.8 ETHICS APPROVAL

Ethics approval was obtained from the University of Warwick Biomedical Science Research Ethics Committee, United Kingdom (REGO-2014-711) (Appendix 7), and the Benue State University Teaching Hospital Health Research Ethics Committee in Nigeria (NHREC/08/11/2013B) (Appendix 8).

16.9 AN ACCOUNT OF THE FIELDWORK PROCESS

I arrived at the Benue State University Teaching Hospital in Makurdi, Nigeria, on the 27th day of August in 2014, and attended a pre-scheduled meeting with the director of the hospital, who also doubled as my supervisor on ground. The main purpose of this meeting was to introduce me to the clinician in charge of the hospital's HIV clinic and obtain formal access to the patients. On the 28th day of August, I began recruiting volunteer research assistants who would administer the questionnaires to the participants. To reiterate, there were four questionnaires to be administered to each participant: The World Health Organisation STEPS tool, the Pittsburgh Sleep Quality Index questionnaire, the Centre for Epidemiologic Studies Depression Scale questionnaire, and the Short Form-12 questionnaire. By the next working day (1 September 2014), I had recruited five volunteer research assistants, all of whom were ad hoc staff of the hospital. Having explained the purpose and scope of the study, as well as the assessments to be carried out on each study participant, I proceeded to conduct training sessions — one hour every work day throughout the week — to educate the research assistants on how to administer the questionnaires. Essentially, I administered each questionnaire to the volunteers as a group,

and had each volunteer administer each questionnaire amongst themselves subsequently, while I observed for any errors. Ialso instructed the volunteers on how to address certain issues raised by the patients during the participant recruitment process; this was achieved by reviewing the participant's information leaflet with the research assistants (Appendix 9).

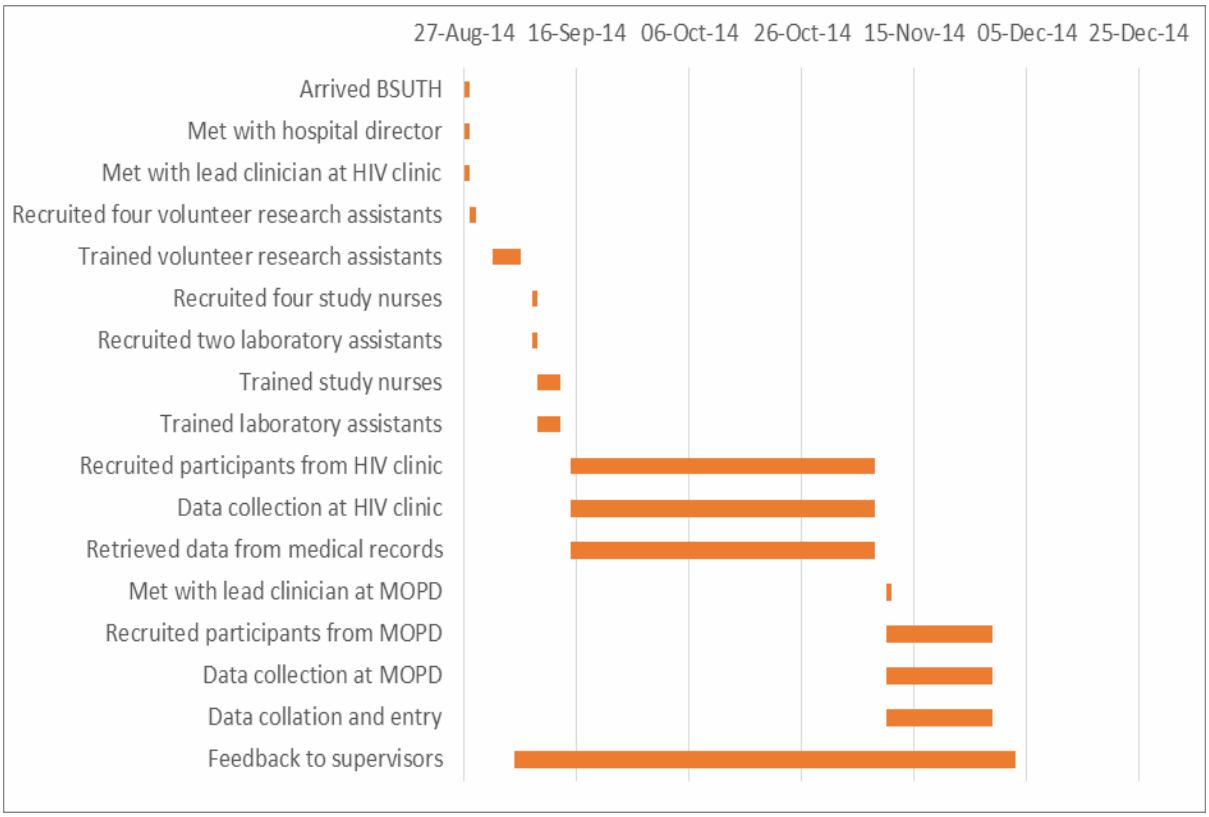
In the second week (taking count from the first day in September 2014), I recruited four study nurses and two laboratory assistants; the purpose and scope of the study were also explained to them. The study nurses were members of the hospital staff who were recruited to conduct physical examinations on the study participants. To reiterate, these examinations entailed blood pressure, weight, height, and waist circumference measurements. During this period, the study nurses were familiarized repeatedly with the blood pressure devices to be used for the study, as well as the standard protocol for each physical examination. The laboratory assistants were employed phlebotomists within the hospital, who were recruited to obtain capillary blood samples for random blood glucose estimation. The phlebotomists were also familiarized repeatedly with the blood glucose monitors to be used in the study, as well as the standard guidelines for capillary blood sampling.

All participant recruitment and data collection were carried out from weeks three to thirteen. Patients were recruited during their follow-up visits to the clinic. Typically, patients attending their follow-up visits at the clinic are encouraged to present early for a short seminar just before opening time. These seminars were forums held daily, where members of the clinic staff would have talks with all presenting patients just before their appointments. The seminars entailed scheduled regular talks about health and social

issues that pertain to living with HIV, and served as the appropriate avenues to communicate the overall purpose of the study to the patients initially. At this point, patients who indicated interest in participating in the study were selected for further dialogues with the volunteer research assistants, who discussed the participant information leaflet with each patient until it was clear that they understood the scope of the study in its entirety. The volunteer research assistants also obtained signed informed consents from patients who would subsequently consent to participate in the study. On average, 10 participants were recruited every work day. Participant recruitment was conducted simultaneously with data collection. In addition to administering questionnaires, data collection also entailed retrieving relevant information from the patients' medical records at the end of each day. The fieldwork was organised in three stations, so that participants proceeded to the second station where they were physically examined by the study nurses, and the third station where their blood samples were obtained for blood glucose estimation. HIV-infected participants in the study were recruited over a period of about eight weeks.

In the following three weeks, the same volunteer research assistants, study nurses and laboratory assistants were involved with the recruitment and collection of data from HIV-negative individuals attending their follow-up visits at the medical outpatients department. All data collection were completed on the 28th day of November 2014. All data obtained were collated and reviewed with the supervisors on a weekly basis. Figure 16.2 is a graphical illustration showing a timeline of the activities carried out during the fieldwork.

Figure 16.2: Gantt chart showing timeline for activities carried out during fieldwork



BSUTH, Benue State University Teaching Hospital; MOPD, medical outpatient department clinic

16.10 STATISTICAL ANALYSES

All data were analysed using Stata version 14 for Windows (Stata Corp, College Station, Texas). Prior to data analysis, the data entered into the Stata database were cleaned by deleting invalid cases and values (including duplicates) within variables. All null hypotheses were tested against two-sided alternative hypotheses at significance level of 5%. The effect estimates were reported with their corresponding 95% confidence intervals.

16.10.1 Descriptive statistics

For all continuous variables (e.g. age, body mass index, waist circumference, sleep duration, systolic blood pressure, diastolic blood pressure, blood glucose concentration,

alcohol consumption in units per week, physical activity in minutes per week, PSQI score, CES-D score, monthly household income, SF-12 physical and mental component scores, and number of fruit and vegetable servings), the means with standard deviations were compared between HAART-exposed and HAART-naïve HIV-infected patients, and HIV-negative individuals. The means (with standard deviations) for CD4 cell count, baseline systolic blood pressure, baseline diastolic blood pressure, and duration of HIV infection were compared between HAART-exposed and HAART-naïve HIV-infected patients only. Mean HAART duration (with standard deviation) was reported for HAART-exposed patients. Statistical significance of the comparisons was determined using the independent samples *t*-test.

Similarly, the frequencies and proportions of categorical variables (e.g. gender, age group, educational attainment, occupational grade, drinking status, smoking status, alcohol consumption categories, physical activity categories, PSQI categories, depression status, overweight/obesity status, central obesity status, family history of hypertension, diabetes mellitus status, and hypertension status) were compared between HAART-exposed and HAART-naïve HIV-infected patients, and HIV-negative individuals. For categorical variables, statistical significance of the comparisons between these patient groups were determined using the chi-squared test or Fisher's exact test where necessary. Differences in baseline characteristics between HAART-exposed, HAART-naïve and HIV-negative patients were also computed across the trend.

16.10.2 Unadjusted analyses

The differences in mean systolic and diastolic blood pressure stratified by HAART status were assessed using one-way analysis of variance (ANOVA). The differences in mean

systolic and diastolic blood pressure stratified by subgroups of other categorical independent variables were also tested using one-way ANOVA. For these unadjusted analyses, one-way ANOVA was appropriate because it is used to test the differences in the means of a continuous dependent variable between two or more subgroups of a categorical independent variable (University of California Los Angeles [UCLA] Statistical Consulting Group, 2016a; Welch, 1951). The one-way ANOVA test is also robust to the normality assumption (UCLA Statistical Consulting Group, 2016a). Statistical significance for each analysis was set at $P < 0.05$.

Simple linear regression models were fitted to test whether there were statistically significant linear relationships between each continuous independent variable and blood pressure. Simple linear regression was the appropriate statistical test for these analysis because it is used when the dependent and independent variables are continuous. Each linear regression model was also fitted separately for HIV-positive and HIV-negative participants in order to identify factors associated with systolic and diastolic blood pressure changes in each group.

16.10.3 Model fit statistics — predictive modelling of hypertension in HIV-infected patients

Improved prediction has been considered essential to causation (Allison, 2014; Cox, 2012), so that it was worthwhile to examine whether HAART status improved the clinical prediction of high blood pressure in HIV-infected patients. To examine whether or not HAART status improved the clinical prediction of hypertension in HIV-infected patients, the best parsimonious model for predicting hypertension in this high-risk group without considering HAART status was fitted. First, simple logistic regression models were fitted to individually assess the best predictors of hypertension. Nagelkerke's R^2 statistic for

each regression model was used to assess how much each variable explained the variability of the data (i.e. the extent of fit of each model), unlike goodness of fit tests which only examine whether or not a model fits the distribution of the given data (UCLA Statistical Consulting Group, 2016b). Nagelkerke's R^2 was used to guide the author on the order of inclusion of the predictor variables in the predictive model. With the exception of HAART status, variables were added in descending order of the magnitude of R^2, such that the predictor variable with the largest R^2 was added first, followed by the predictor variable with the second largest R^2 and so on. Based on R^2, the stopping rule was a failure to improve model fit following addition of the subsequent variable to the predictive model. Once the predictive model was fitted, HAART status was added to evaluate its impact on the predictive value of the model.

Given that any observed improvement in model fit following the addition of HAART status essentially reflects the adjusted difference in hypertension risk between HAART-exposed and HAART-naïve patients, predictive margins were computed to determine the predicted probabilities of hypertension in HAART-exposed and HAART-naïve patients at different fixed values of the (other) covariates in the predictive model. The predicted probabilities of hypertension were also illustrated graphically. The estimated probabilities of hypertension for HAART-exposed and HAART-naive patients were reported based on the final prediction model. Assuming all patients in the study were HAART-naïve, the predictive margin was used to estimate the predicted probability of hypertension for each HAART-naïve patient and the average for all patients was calculated (Graubard & Korn, 1999). The probability of hypertension for HAART exposed patients was similarly obtained where, assuming all patients in the study were exposed to HAART, the predicted probability of hypertension for each HAART-exposed patient and the average for all patients were calculated.

The predictive model accuracy was measured — before and after including HAART status — by computing the area under the receiver operating characteristics (ROC) curve. The ROC curve plots the sensitivity of the model in predicting hypertension against (1 minus its specificity). The greater the area under the ROC curve (upper limit = 1), the better the model discriminates between hypertension cases.

16.10.4 Mediation analysis — a structural equation modelling approach

Structural equation models were fitted to test whether there were alternative causal pathways between HAART and blood pressure that were mediated by changes in body fat measures (e.g. body mass index and waist circumference), blood glucose concentration, sleep quality indices, or the presence of depressive symptoms. To recall, the aim is to test the hypotheses that HAART exposure precedes changes in body mass index, waist circumference, blood glucose concentration, sleep quality indices, and depression scores, which, in turn, precede changes in blood pressure. In other words, controlling for these intervening variables should reduce or eliminate the differences in blood pressure between HAART-exposed and HAART-naïve HIV-infected patients. Although, effect-modification (or moderation), which occurs when the explanatory variable (i.e. HAART status) has a different effect on the dependent variable (blood pressure) at different values of the effect-modifier, may also explain how the sequential steps of an exposure lead to its outcome; however, mediation analyses are more appropriate and more powerful to test causal hypotheses (Kenny, 2015). In fact, the findings of a mediation analysis are only valid if they are consistent with a causal hypothesis that is theoretically plausible (Judd & Kenny, 2010; Kenny, 2015).

Hyman (1955), MacCorquodale & Meehl (1948), and Wright (1934) were among the landmark studies that conceptualized the use of mediation to estimate alternative causal pathways between an exposure and an outcome. More recently, Kenny (2015) described the mediation model as a causal model, such that the causal variable is presumed to cause the mediator, which is, in turn, presumed to cause the outcome. Where the reverse is also the case, the mediation model is not likely to be correctly specified, which potentially invalidates the results from the mediation analysis. In other words, even though mediation analyses are appropriate to test causal assumptions between an exposure and an outcome, the results from such analyses are only valid if the presumed mediational model is correctly specified.

Historically, mediation analyses have been performed using standard regression techniques. Baron and Kenny (1986), James and Brett (1984), and Judd and Kenny (1981) outlined four regression steps to ensure that a mediation model was correctly specified:

- Step I: to show that there is an association between the causal variable (HAART status) and the dependent variable (blood pressure). This equation entails regressing the dependent variable on the causal variable.
- Step II: to show that there is an association between the causal variable and each mediator variable (body mass index, waist circumference, blood glucose concentration, sleep quality indices, and depression scores). This equation entails regressing the mediator variable on the causal variable.
- Step III: to show that there is an association between the mediator variable and the dependent variable, independent of the causal variable. This step entails regressing the dependent variable on the mediator variable, while adjusting for the causal variable.

- Step IV: to show that the mediator variable completely attenuates the effect of the causal variable on the dependent variable. This step entails regressing the dependent variable on the causal variable, while controlling for the mediator variable.

The mediating effect was *complete* when all four steps were satisfied, and *partial* when only the first three steps were satisfied. Nonetheless, satisfying these conditions did not invariably mean that mediation (complete or partial) had occurred. Judd & Kenny (1981) affirmed that the presence of *specification errors* in the mediation model, notably the potential for confounding and reverse causation, could invalidate the findings of a mediation analysis. Such specification errors had to be investigated and corrected before any causal inferences could be made about a mediational model — a process described many years later as the *causal inference* approach to mediation (Robins & Greenland, 1992). Robins and Greenland (1992) were among the earliest proponents of the causal inference approach, which aims to provide a valid basis for making causal inferences about the results of a mediation analysis. The approach was predicated on the condition that there were no unmeasured factors that confounded the indirect effects of the causal variable on the outcome through the mediator in each of the previously outlined steps required for model specification.

While the causal inference approach still remains relevant in mediation analysis, there is a growing interest in the use of structural equation modelling — as opposed to standard regression methods — to provide a more suitable inference framework for mediation analyses, as well as for other types of causal analyses (Gunzler *et al*., 2013). Structural equation modelling (SEM) has been described as "a very powerful multivariate technique that entails a conceptual model, path diagram and system of linked regression-style

equations to capture complex and dynamic relationships within a web of observed and unobserved variables" (Gunzler *et al*., 2013; p. 390). The technique was conceptualized by geneticist Wright (1921) and economists Haavelmo (1943) and Koopmans (1953) who sought to estimate and test hypotheses on cause-effect relationships among a set of associated variables. In SEM, the clear distinction between independent and dependent variables — as is the case with standard regression models — is relative in the sense that a dependent variable in one model equation can become an independent variable in other component equations of the structural equation model: a concept that makes SEM better-suited to infer causal associations than standard regression techniques (Gunzler *et al*., 2013).

Another advantage of using SEM over standard regression methods for mediation analysis is that the former is user-friendly and allows for the estimation and interpretation of complicated mediational models in a single analysis (MacKinnon, 2008); whereas, standard regression methods rely on combining results from two or more equations. Furthermore, "while standard regression methods test a statistical relationship based on a conditional expected value, SEM tests a functional relationship expressed via a conceptual model, path diagram, and mathematical equations. Thus, the causal relationships in a hypothesized mediation process, the simultaneous nature of the indirect and direct effects, and the dual role the mediator plays as both a cause for the outcome and an effect for the intervention are more appropriately expressed using structural equations than using regression analysis" (Grunzler *et al*., 2013; p. 391).

For each structural equation model, the direct effects of HAART on systolic and diastolic blood pressure, and the indirect effects of HAART on systolic and diastolic blood pressure through each potential mediator, were computed; the coefficients of the indirect effects correspond to the mediation coefficients. The structural equation model also computed the total effects of HAART on systolic and diastolic blood pressure, which were the sums of each direct and indirect effect. To further clarify the difference between the total and direct effects: the total effects were the effects of HAART on systolic and diastolic blood pressure unadjusted for any potential mediator; whereas, the direct effects were the effects of HAART on systolic and diastolic blood pressure after adjusting for the proposed mediator in the structural equation model (Rucker *et al*., 2011). The amounts of the effects of HAART on systolic and diastolic blood pressure that were explained by each mediator were derived by computing the ratios of each indirect effect to the total effect.

In line with the causal inference approach to mediation, the mediation models were subsequently fitted to adjust for common potential confounders (or omitted variables), including traditional risk factors (age, sex, smoking status, drinking status, physical activity) and HIV-related characteristics (CD4 cell count and duration of HIV infection). The rationale for selecting potential confounders were based on potential interactions with HAART exposure, blood pressure and each potential mediator as described below:

- *Age*: Evidence suggests that older antiretroviral-naïve HIV-infected patients may be more likely than their younger counterparts to commence HAART given the impact of age on HIV disease progression (Edwards *et al*., 2015). In addition, there is an age-related increase in body mass index (Mariz *et al*., 2011), blood glucose concentration (Shen *et al*., 2013) and blood pressure (Palacious *et al*., 2006) among people living with HIV.

Although the effect of ageing on sleep quality in people living with HIV remains largely unknown, sleep quality in the general population tends to be characterised by an age-related decline (Pace-Schott & Spencer, 2011).

- *Sex*: Women are less likely than men to have access and optimal adherence to antiretroviral therapy (Tapp *et al*., 2011). Gender differences exist in central fat accumulation among people living with HIV, with women being more affected than men (Joy *et al*., 2008). In a sample comprising 2,006 HIV-infected patients, prevalence estimates of hyperglycaemia were found to be higher among men than women [21.5% *versus* 15.2%]) (Shen *et al*., 2013). Men were significantly less likely than women to have sleep disturbances in a sample of 1,354 HIV-infected participants (Allavena *et al*., 2014). HIV- infected men are more likely than HIV-infected women to have high blood pressure (Thiebaut *et al*., 2005).
- *Lifestyle factors*: Smoking and alcohol abuse have been linked to antiretroviral non-adherence, which precludes the essence of HAART exposure (Shuter & Bernstein, 2008; Chander, Lau & Moore, 2006). Among people living with HIV, smoking is associated with a decreased risk in overweight and obesity, whereas alcohol consumption is associated with a decreased risk of underweight (Mariz *et al*., 2011). Tobacco smoking and alcohol consumption have been linked to sleep disturbances in people living with HIV (Allavena *et al*., 2014). Smoking and alcohol consumption are risk factors for high serum glucose levels and high blood pressure in people living with HIV (Dau & Holodniy, 2008; Klatsky, 1995). Low physical activity levels have been linked to antiretroviral non-adherence which may rule out the essence of HAART exposure (Blashill *et al*., 2013). There is a significant negative correlation between physical activity levels and waist circumference in people living with HIV (Florindo *et al*., 2007). While the role of physical activity in sleep quality has rarely been studied among people living with HIV, physical

activity may improve the quality of sleep — and vice versa — in the general population (Zuo *et al*., 2012). Physical inactivity is a known predisposing factor for hyperglycaemia and type II diabetes mellitus. Physical activity levels below 150 minutes per week is associated with endothelial dysfunction, which impairs blood pressure regulation (Dirajlal-Fargo *et al*., 2015).

- *CD4 cell count*: Naturally, CD4 cell counts increase following HAART. Low CD4 cell counts below 200 cells/mm^3 have been associated with reduced relative weight (Mariz *et al*., 2011), worsening quality of sleep (Oshinaike *et al*., 2014), increasing blood glucose concentrations (Shen *et al*., 2013), and sustained high blood pressure (Manner *et al*., 2013).
- *HIV infection duration*: Prolonged duration of HIV infection has been identified as a significant contributor to high blood pressure (Manner *et al*., 2010; Manner *et al*., 2012), increased body mass index (Crum-Cianflone *et al*., 2010), altered glucose metabolism (Kalra *et al*., 2011), and poor sleep quality (Allavena *et al*., 2014; Oshinaike *et al*., 2014).

As part of the causal inference approach to mediation, the author also sought to rule out reverse causation and any alternate models that may potentially invalidate causal inferences about the mediational models (Kenny, 2015).

Bootstrap tests of mediation with 95% confidence intervals were subsequently performed using 500 replications to determine whether the indirect effects were statistically significant. This non-parametric test — unlike the Sobel-Goodman test — is not affected by sample size, so that the relatively small size of the present study was unlikely to produce inaccurate estimates of the indirect effects (Preacher & Hayes, 2008;

MacKinnon, Lockwood & Williams, 2004). The 95% confidence intervals of the indirect effects were bias-corrected to allow for potential skewness in the data (Kenny, 2015).

16.10.5 Propensity score matching

A propensity score model was fitted to estimate a balanced distribution of propensity scores between HAART-exposed and HAART-naïve participants. The propensity score model is much like a logistic regression model where a dichotomous variable, which is the treatment status (or exposure criterion), is regressed on a number of observed baseline covariates (Austin, 2011a). Subsequently, a propensity score *matching* model — now including the study outcomes and a variable denoting the propensity score — was fitted to examine the causal average effects of antiretroviral therapy on systolic and diastolic blood pressure.

Propensity score matching entailed using nearest neighbour matching with a calliper width of 0.2, which has been recommended as most suitable for observational studies in which the effect estimates reflect the mean differences (Austin, 2011b). Austin (2011b) also argued that such a matching specification offers the best chance of achieving a 'variance-bias trade-off' in which the specified caliper is not so wide as to allow systematic differences between matched treated and untreated patients, thereby introducing bias in the average treatment effect, and not so narrow that it substantially reduces the number of matched patients, thereby increasing the variance of the average treatment effect. A good overall matching performance was defined not only in terms of the differences in means and proportions of the baseline covariates between HAART-exposed and HAART-naïve patients in the propensity score matched sample, but also in terms of the standardized bias after matching (Rosenbaum & Rubin, 1985), and a comparison of the variances of

continuous variables between the matched treatment groups (Austin, 2011b). A standardized bias (or mean absolute bias) of 5% or less after propensity score matching indicated a good balance of baseline covariates between patients naïve and exposed to HAART (Grilli & Rampichini, 2011). Similarly, the variance ratios of the continuous covariates had to be below the upper limit of the range determined by the statistical software (Austin, 2011b). Based on these definitions, the balancing of the variables between HAART-exposed and HAART-naïve patients was checked using Stata's *pstest* command after propensity score matching in order to be sure that the estimated treatment effect was the result of a successfully matched model.

Given that the target population was people living with HIV in a sub-Saharan African setting, propensity score matching was limited to matching the baseline characteristics of HAART-exposed and HAART-naïve participants on the estimated propensity scores. Evidently, estimating propensity scores for HIV-negative individuals was considered redundant with respect to investigating the possibility of a causal association between antiretroviral therapy and increased blood pressure in people living with HIV.

The term 'propensity score' was originally defined as "the conditional probability of assignment to a particular treatment given a vector of observed covariates" (Rosenbaum & Rubin, 1983; p. 41). However, Austin (2011a) explained it more clearly as a balancing score conditional on which the distribution of baseline characteristics is similar between patients exposed and naïve to a treatment — much like a randomised controlled trial. "The propensity score exists in both randomized experiments and in observational studies" (Austin, 2011a; p. 403). In a randomised controlled trial, the true propensity score is determined by the randomization process. While the true propensity score is not known

in an observational study, it can be estimated using the study data (Austin, 2011a). Other similarities between the analysis of a propensity score-matched sample and that of a randomised controlled trial (Austin, 2011a; Imbens, 2004; Rubin & Thomas, 2000; Steyerberg, 2009) are summarised in Table 16.1 below.

The idea of the propensity score originated from the need to examine cause-and-effect relationships in cases where controlled experiments were not feasible (Rosenbaum & Rubin, 1983). According to Cochran and Chambers (1965), the main objective of an observational study is to estimate causality, same as controlled experiments, putting into perspective the growing interest in using observational data for this intended purpose, as well as the importance of propensity score methods. To reiterate, controlled experimentation may not suffice to determine whether or not a causal effect exists between antiretroviral therapy and increased blood pressure, as randomising HIV-infected patients to a control group where they are administered placebos would pose considerable ethical constraints. In addition, any attempt to ensure that the control group comprises only HIV-infected patients who are not eligible to commence antiretroviral treatment biases the randomisation process. According to the HIV/AIDS care and treatment guidelines in Nigeria, HIV-infected patients are only considered eligible to commence antiretroviral therapy when they are in the advanced clinical stages III and IV of HIV infection or CD4 cell counts are below 350 cells/mm^3 (Federal Ministry of Health Nigeria, 2010). Hence, the possibility that the treatment and control groups will comprise HIV-infected patients for whom antiretroviral therapy is indicated and contra-indicated respectively, would be impossible to achieve by randomisation. Moreover, in the highly unlikely event that the randomisation process results in this 'desired' treatment allocation, substantial differences in baseline characteristics are bound to exist between both groups

of patients, and the potential for confounding, as well as selection bias, is bound to be high. These hypothetical scenarios are inevitable, potentially underscoring the need for propensity score methods in the present observational study.

Besides propensity score matching, other propensity score methods include: stratification on the propensity score, which would entail stratifying the study participants according to quantiles of the estimated propensity scores; and covariate adjustment using the propensity score, which would entail regressing each of the outcome variables (systolic and diastolic blood pressure) on HAART status and a third variable denoting the propensity score (Austin, 2011a; Austin & Mamdani, 2006; Rosenbaum, 1987; Rosenbaum and Rubin, 1983). Among these propensity score methods, propensity score matching was the preferred option used in the present study because it eliminates a greater amount of the baseline differences between treatment-exposed and treatment-naïve subjects (Austin, 2009; Austin, Groontendorst & Anderson, 2007; Austin & Mamdani, 2006).

Table 16.1: Similarities between the analyses of a propensity score-matched sample and a randomised controlled trial

Analysis of propensity score-matched sample	Analysis of randomised controlled trial
Conditional on the propensity score, the propensity score-matching process ensures a balanced distribution of the baseline characteristics between treated (or exposed) and untreated (unexposed) patients.	The randomisation process ensures a balanced distribution of the baseline characteristics between treated and untreated subjects.
The similarity in baseline characteristics between treated and untreated patients allows for estimation of the average treatment effect on the treated (ATT).	The absence of any baseline differences between treatment groups also allows for estimation of the average treatment effect (ATE)
Residual differences may exist between treated and untreated patients even after propensity score matching.	Residual differences may also exist between treatment groups even after randomisation.
Bias due to residual baseline differences between treated and untreated patients can be reduced by using other propensity score matching specifications or by combining propensity score matching with regression adjustment.	Regression adjustment can also be used to reduce bias due to residual differences in the baseline characteristics between treatment groups.

16.10.5.1 The average treatment effect on the treated (ATT)

In randomised controlled trials, the estimated effect of the intervention on the outcome is the average treatment effect (ATE). Hence, the ATE measures the difference in the outcome measure between subjects assigned to the treatment and subjects assigned to the control. Austin (2011a; p. 401) defined the ATE as "the average effect, at population level, of moving an entire population from untreated to treated"

Closely related to the ATE is the average treatment effect on the treated (ATT or ATET), which is the average effect of the treatment (or exposure) on those subjects who were actually treated (or exposed) (Austin, 2011a; Imbens, 2004). It may appear that the difference between the ATE and ATT lies with the respective target populations: a randomly selected sample of untreated patients at baseline — as is often the case with randomised controlled trials — *versus* a population sample including patients already treated at baseline — as is the case with observational studies. However, both measures of treatment effects are valid only when the treatment is randomly assigned conditional on a balanced distribution of baseline characteristics. Both treatment effect measures also entail a statistical approach to defining causality based on the Rubin causal model, which holds that the causal effect of being exposed to a treatment, as opposed to being unexposed to the same treatment at any given time, is the difference between the outcome measures with and without treatment (Jasjeet, 2007; Rubin, 1974).

For instance, with the treatment status being a dichotomous variable:

$ATE = E\,[Y_1 - Y_0]$,

Where Y_1 is the outcome with treatment, Y_0 is the outcome without treatment;

$ATT = E\,[Y_1 - Y_0 \mid W = 1]$,

Where Y_1 is the outcome with treatment, Y_0 is the outcome without treatment, and W is the dichotomous treatment indicator where ($W = 1$) denotes 'treatment' and ($W = 0$) denotes 'no treatment' (Rubin, 1974).

It is worth emphasizing that while the ATE and ATT are often measured as the estimated treatment effects in experimental and observational studies respectively, this 'rule' remains flexible, as the ATE may be of greater utility and interest in estimating the average effect of a treatment or exposure in an observational study and vice versa (Austin, 2011a). However, regardless of the context of the research, propensity score matching is reserved for estimating the ATT (Austin, 2011a; Imbens, 2004).

16.10.6 Sample size calculations

16.10.6.1 Sample size estimation for mediation analysis

Recall that mediation analysis was performed using the SEM approach. Although there is little consensus on the recommended sample size for SEM (Sivo *et al.*, 2006), a 'critical sample size' of 200 was proposed by Garver & Mentzer (1999) and Hoelter (1983). Hence, as a rule of thumb, any sample size above 200 is generally understood to provide sufficient statistical power for SEM analysis.

However, Fritz and MacKinnon (2007) argue that the mediation model is essentially a linear model, so that sample size calculation to evaluate mediation analysis for such a model be given by the following equation:

$$n = \frac{L}{f^2} + k + 1$$

Where *n* denotes the sample size.

L = 7.85, which is the linear statistic corresponding to ordinary least squares (OLS) regression for one predictor with a type I error of 0.05 and power of 0.8.

k = 1, which is the number of predictors in each OLS regression model.

f is the coefficient of the regression equation corresponding to the indirect effect of the predictor variable on the outcome variable, through the mediator.

Drawing on Cohen's criteria for small effect sizes, it was assumed that the β coefficients for the association between the exposure (HAART) and any of the potential mediators, and the association between any of the potential mediators and the outcome (blood pressure) were 0.14 each (Cohen *et al*., 2003). Hence, the indirect effect (f) would entail the product of both β coefficients (i.e. 0.0196).

Substituting these default values in the equation above yielded a sample size (*n*) of 403.

16.10.6.2 Sample size estimation for propensity score matching

Given that more than a few baseline characteristics of HAART-exposed and HAART-naïve participants were to be matched on the propensity score, a substantial proportion of patients — who were not matched on the propensity score — were likely to be dropped from the analysis (Schneeweiss, 2010). Consequently, the sample size calculation accounted for a hypothetical proportion of patients who were dropped out from the propensity score analysis (Velentgas *et al*., 2013). Recall that HIV-negative individuals were not assigned propensity scores, so that the sample size was given by:

$$n = \frac{2\,(Z_{\alpha/2} + Z_{\beta})^2}{\delta^2}.$$

As described in the statistical analysis section, two-sided tests at 5% significance were performed, and for such tests, $Z_{\alpha/2} = 1.96$. A sample size that achieved 90% power, which corresponds to $Z_{\beta} = 1.28$, was desired. The study was powered to detect a standardised mean difference (δ) of 0.3mmHg in systolic blood pressure between HAART-exposed and HAART-naïve participants based the meta-analysis of the association between antiretroviral therapy and systolic blood pressure (Nduka *et al*., 2015b). Hence, accounting for 25% drop out of patients who were not matched on the propensity score (Velentgas *et al*., 2013), the estimated sample size of the propensity score matched sample was 293.

CHAPTER SEVENTEEN

RESULTS – SAMPLE DESCRIPTION

In this chapter, I describe the characteristics of participants included in the primary study.

17.1 SAMPLE DESCRIPTION

A total of 512 participants — 306 HAART-exposed HIV-infected patients, 100 HAART-naïve HIV-infected patients, and 106 HIV-negative individuals — were enrolled into the study. Overall, the mean (± SD) age of the study population was 37.6 ± 11.2 years, with participants' age ranging from 18 to 88 years old. A majority (66%) of the participants were aged between 18 and 40 years. Women accounted for 64% of the study population. About 72% of the participants were either members of the working class or unemployed; 45% had progressed beyond a formal secondary education. Approximately 11% had ever smoked cigarettes and 28% were current alcohol consumers. Among the 142 participants who were current alcohol users, 21% were heavy drinkers, consuming more than 21 units of alcohol in a typical week. Of note, men were more than twice as likely to be heavy drinkers, compared to women.

The clinical characteristics of the study population are summarised as follows: hypertension was diagnosed in 104 (20%) of 512 participants, including 17 individuals who were on antihypertensive treatment; 58% of all patients diagnosed with hypertension were HIV-infected; mean body mass index was 24.7 ± 4.6 kg/m^2, with 41% of the participants identified as overweight or obese; mean waist circumference was 85.9 ± 11.6 cm, with 43% of the study participants identified as centrally obese; mean random blood glucose concentration was 5.2 ± 2.0 mmol/L, including a total of six participants (1%) with random blood glucose levels above 11.1 mmol/L, and ten participants (2%) on

treatment for type 2 diabetes mellitus; severe depressive symptoms (CES-D score ≥ 16) were found in approximately 19% of participants; 36% reported poor quality of sleep (PSQI ≥ 5); and 12.5% had a positive family history of hypertension. Among HIV-infected patients, the mean duration of HIV infection was 45.0 ± 29.4 months; mean duration of HAART was 45.6 ± 27.9 months; mean CD4 count was 454 ± 251 cells/mm^3, with CD4 counts above 350 cells/mm^3 in 64% of patients, and above 200 cells/mm^3 in 86% of patients; opportunistic infections or AIDS-defining illnesses were absent.

Table 17.1 summarises other socio-demographic and clinical data in HAART-exposed, HAART-naïve, and HIV-negative individuals. As shown, there were notable differences across the three participant groups in sex distribution, socio-economic status, lifestyle factors, relative weight, and random blood glucose concentration. For instance, HIV-infected participants in the HAART-naïve group were more likely to be women (78.0% *versus* 65.4% and 48.1%; $P < 0.001$ for trend), members of the working class or unemployed (91.9% *versus* 68.1% and 65.7%; $P < 0.001$ for trend), and less educated (81.0% *versus* 52.5% and 38.7%; $P < 0.001$ for trend), compared to participants in the HAART-exposed and HIV-negative groups respectively. Likewise, HIV-negative individuals were more likely than HAART-exposed and HAART-naïve HIV-infected patients to have larger body mass indices (mean ± SD = 26.7 ± 5.0 kg/m^2 *versus* 24.5 ± 4.4 kg/m^2 and 23.4 ± 4.3 kg/m^2; $P < 0.001$ for trend), larger waist circumferences (mean ± SD = 87.4 ± 13.7 cm *versus* 86.3 ± 10.3 cm and 82.9 ± 12.2 cm; $P = 0.002$ for trend), higher blood glucose concentrations (mean ± SD = 5.9 ± 2.3 mmol/L *versus* 5.1 ± 2.1 mmol/L and 4.8 ± 1.3 mmol/L; $P < 0.001$ for trend), higher prevalence estimates of hypertension (42.4% *versus* 16.3% and 9.0%; $P < 0.001$ for trend), less likely to be physically active (mean ± SD = 122.3 ± 136.6 minutes/week *versus* 150.3 ± 166.3

minutes/week and 222.5 ± 188.5 minutes/week; $P < 0.001$ for trend), more likely to be current drinkers (39.6% *versus* 24.5% and 25.0%; $P = 0.009$ for trend), and more likely to have been on antihypertensive treatment within the past two weeks (9.4% *versus* 1.6% and 2.0%; $P < 0.001$ for trend). Among these characteristics, differences observed by sex distribution ($P = 0.018$), occupational grade ($P < 0.001$), educational attainment ($P = 0.028$) and level of physical activity ($P = 0.001$) were driven by comparisons between HAART-exposed and HAART-naïve HIV-infected patients.

CD4 cell counts were significantly higher among HAART-naïve patients, compared to HAART-exposed patients (mean ± SD = 504 ± 227 cells/mm^3 *versus* 440 ± 256 cells/mm^3; $P = 0.006$), so that it was not unexpected that the mean duration of HIV infection was longer in HAART-exposed patients, compared to HAART-naïve patients (mean ± SD = 50.2 ± 29.3 months versus 29.0 ± 22.8 months; $P < 0.001$). At the time of HIV infection diagnosis, systolic and diastolic blood pressure readings were not significantly different between patients now classified as naïve and exposed to HAART.

Table 17.1: Characteristics of the study sample

Characteristics	HAART-exposed (N = 306)	HAART-naïve (N = 100)	*P* value (exposed *vs* naïve)	HIV-negative (N = 106)	*P* value for trend
Age (years), mean ± SD	38.56 ± 9.99	35.63 ± 12.48	0.586	36.67 ± 12.77	0.449
Age group (years), n (%)					
18–40	189 (62.6)	75 (76.5)		69 (65.1)	
41–64	108 (35.8)	20 (20.4)		36 (34.0)	
≥65	5 (1.6)	3 (3.1)	0.015	1 (0.9)	0.060
Gender, n (%)					
Female	200 (65.4)	78 (78.0)		51 (48.1)	
Male	106 (34.6)	22 (22.0)	0.018	55 (51.9)	0.000
Occupational grade, n (%)					
Middle class	97 (31.9)	8 (8.1)		36 (34.3)	
Working class/unemployed	207 (68.1)	91 (91.9)	0.000	69 (65.7)	0.000
Educational status, n (%)					
≤ 12 years	160 (52.5)	81 (81.0)		41 (38.7)	
> 12 years	145 (47.5)	19 (19.0)	0.028	65 (61.3)	0.000
Smoking status, n (%)					
Never smoker	266 (89.3)	88 (89.8)		94 (88.7)	
Ever smoker	32 (10.7)	10 (10.2)	0.295	12 (11.3)	0.967
Drinking status, n (%)					
Abstainer	231 (75.5)	75 (75.0)		64 (60.4)	
Drinker	75 (24.5)	25 (25.0)	0.397	42 (39.6)	0.009
Physical activity (mins/wk), mean ± SD	150.3 ± 166.3	222.5 ± 188.5	0.001	122.3 ± 136.6	0.000

Characteristics	HAART-exposed (N = 306)	HAART-naïve (N = 100)	*P* value (exposed *vs* naïve)	HIV-negative (N = 106)	*P* value for trend
Physical activity (minutes/week), n (%)					
< 150	187 (61.5)	46 (46.0)		74 (69.8)	
≥ 150	117 (38.5)	54 (54.0)	0.007	32 (30.2)	0.002
Body mass index (kg/m^2), mean ± SD	24.5 ± 4.4	23.4 ± 4.3	0.931	26.7 ± 5.0	0.000
Body mass index (kg/m^2), n (%)					
< 25	175 (59.7)	70 (71.4)		45 (43.7)	
≥ 25	118 (40.3)	28 (28.6)	0.038	58 (56.3)	0.000
Waist circumference (cm), mean ± SD	86.3 ± 10.3	82.9 ± 12.2	0.213	87.4 ± 13.7	0.002
Central obesity, n (%)					
No	166 (54.2)	58 (58.0)		69 (65.1)	
Yes	140 (45.8)	42 (42.0)	0.513	37 (34.9)	0.149
RBG (mmol/L), mean ± SD	5.1 ± 2.1	4.8 ± 1.3	0.320	5.9 ± 2.3	0.000
Hyperglycaemia, n (%)					
No	284 (96.6)	92 (95.8)		95 (92.2)	
Yes	10 (3.4)	4 (4.2)	0.726	8 (7.8)	0.180
Hypertension, n (%)					
No	256 (83.7)	90 (90.0)		61 (57.6)	
Yes	50 (16.3)	10 (10.0)	0.071	45 (42.4)	0.000
Antihypertensive treatment, n (%)					
No	282 (92.2)	96 (96.0)		73 (68.9)	
Yes	24 (7.8)	4 (4.0)	0.256	33 (31.1)	0.000
PSQI, mean ± SD	4.1 ± 2.9	4.2 ± 3.2	0.958	3.8 ± 2.8	0.400

Characteristics	HAART-exposed (N = 306)	HAART-naïve (N = 100)	*P* value (exposed *vs* naïve)	HIV-negative (N = 106)	*P* value for trend
PSQI, n (%)					
< 5	191 (63.7)	65 (65.7)		67 (63.2)	
≥ 5	109 (36.3)	34 (34.3)	0.720	39 (36.8)	0.719
CES–D, mean ± SD	9.9 ± 7.4	10.1 ± 7.9	0.423	8.0 ± 6.9	0.054
CES–D, n (%)					
< 16	249 (81.4)	78 (78.0)		89 (84.0)	
≥ 16	57 (18.6)	22 (22.0)	0.460	17 (16.0)	0.275
HRQL score (physical), mean ± SD	50.5 ± 8.0	49.0 ± 8.5	0.171	47.5 ± 10.2	0.377
HRQL score (mental), mean ± SD	51.9 ± 9.3	51.8 ± 9.5	0.861	51.8 ± 8.7	0.673
Family history of hypertension, n (%)					
No	268 (88.5)	89 (89.9)		76 (81.7)	
Yes	35 (11.5)	10 (10.1)	0.457	17 (18.3)	0.165
CD4 cell count (cells/mm^3), mean ± SD	440 ± 256	504 ± 227	0.003	–	–
HIV duration (months), mean ± SD	50.2 ± 29.3	29.0 ± 22.8	0.000	–	–
HAART duration (months), mean ± SD	45.5 ± 28.2	–	–	–	–
Baseline SBP (mmHg), mean ± SD	111.8 ± 15.6	112.7 ± 18.5	0.376	–	–
Baseline DBP (mmHg), mean ± SD	73.6 ± 11.6	74.1 ± 12.3	0.800	–	–

CESD, Centre for Epidemiologic Studies Depression Scale questionnaire; DBP, diastolic blood pressure; HAART, highly active antiretroviral therapy; HRQL, health-related quality of life; PSQI, Pittsburgh Sleep Quality Index; SBP, systolic blood pressure; SD, standard deviation.

CHAPTER EIGHTEEN

RESULTS – UNADJUSTED ANALYSES

In this chapter, I identify factors potentially related to blood pressure in HIV-infected and non-infected persons, having fitted simple linear models.

18.1 RESULTS OF UNADJUSTED ANALYSES – FACTORS RELATED TO BLOOD PRESSURE CHANGES IN HIV-INFECTED PARTICIPANTS.

The results from fitting the simple linear models are presented in Table 18.1. Mean systolic ($P < 0.001$) and diastolic blood pressure ($P < 0.001$) were significantly higher in HAART-exposed patients, compared to HAART-naïve patients. Besides HAART status, other variables with statistically significant differences in mean systolic and diastolic blood pressure between subgroups included: age, sex, educational attainment, occupational grade, drinking status, body mass index (overweight/obesity), family history of hypertension, and a recent history of antihypertensive treatment. Mean systolic blood pressure was highest among HIV-infected patients who were 65 years and above, compared to HIV-infected patients aged 41 to 64 years, and 18 to 40 years) ($P < 0.001$), whereas mean diastolic blood pressure was highest among HIV-infected patients aged 41 to 64 years, compared to those aged 18 to 40 years, and those aged 65 years and above ($P < 0.001$). Mean systolic ($P = 0.005$) and diastolic blood pressure ($P = 0.001$) were higher among men than women. Mean systolic ($P = 0.027$) and diastolic blood pressure ($P = 0.002$) were higher among HIV-infected patients who had completed more than 12 years of formal education, compared to patients who had completed fewer years of formal education. HIV-infected patients who belonged to the middle class had significantly higher mean systolic ($P = 0.038$) and diastolic blood pressure ($P = 0.001$), compared to

HIV-infected patients who were either unemployed or members of the working class. Mean systolic ($P = 0.015$) and diastolic blood pressure ($P = 0.014$) were significantly higher among HIV-infected patients who were current drinkers, compared to patients who abstained from alcohol. Mean systolic ($P < 0.001$) and diastolic blood pressure ($P < 0.001$) were significantly higher among HIV-infected patients who were overweight or obese, compared to patients who were normal weight. HIV-infected patients with a positive family history of hypertension had higher mean systolic ($P < 0.001$) and diastolic blood pressure ($P = 0.001$), compared to those with no family history of hypertension. HIV-infected patients on antihypertensive treatment had significantly higher mean systolic ($P < 0.001$) and diastolic blood pressure ($P < 0.001$), compared to HIV-infected patients not on antihypertensive treatment.

Table 18.1: Group means (± standard deviation) of systolic and diastolic blood pressure in HIV-infected patients.

Factors	Systolic BP (mmHg)		Diastolic BP (mmHg)	
	Mean ± SD	*P* value	Mean ± SD	*P* value
HAART status				
HAART-naïve	112.60 ± 14.33		71.57 ± 8.60	
HAART-exposed	121.77 ± 19.69	0.000	78.80 ± 9.88	0.000
Age group				
18 to 40 years	116.33 ± 14.76		75.08 ± 8.68	
41 to 64 years	125.26 ± 24.41		80.87 ± 11.78	
≥ 65 years	128.94 ± 23.06	0.000	78.13 ± 6.91	0.000
Gender				
Female	117.70 ± 17.42		75.88 ± 9.72	
Male	123.46 ± 21.39	0.005	79.50 ± 10.42	0.001
Education				
≤ 12 years	117.79 ± 16.81		75.75 ± 8.98	
> 12 years	122.08 ± 21.48	0.027	78.91 ± 11.28	0.002
Occupational grade				
Middle-income	122.77 ± 22.19		79.91 ± 10.86	
Working class/unemployed	118.22 ± 17.56	0.038	75.95 ± 9.61	0.001
Smoking status				
Never smoker	119.17 ± 19.35		76.87 ± 10.37	
Ever smoker	121.82 ± 16.71	0.402	75.60 ± 8.30	0.667
Drinking status				
Abstainer	118.14 ± 16.95		76.28 ± 9.50	
Drinker	123.48 ± 23.40	0.015	79.15 ± 11.37	0.014
Physical activity				
< 150 minutes/week	119.56 ± 17.59		77.57 ± 9.42	
≥ 150 minutes/week	119.20 ± 20.61	0.849	76.11 ± 10.77	0.155
Body mass index				
< 25 kg/m^2	116.21 ± 16.59		75.20 ± 9.49	
≥ 25 kg/m^2	124.89 ± 21.42	0.000	79.92 ± 10.43	0.000
Central obesity				
No	118.24 ± 19.47		76.15 ± 10.35	
Yes	120.97 ± 18.19	0.152	78.00 ± 9.61	0.067
Hyperglycaemia				
No	119.13 ± 18.63		76.86 ± 9.83	
Yes	118.79 ± 13.53	0.945	76.21 ± 10.28	0.811
Antihypertensive treatment				
No	118.93 ± 18.18		76.75 ± 9.85	
Yes	150.64 ± 31.83	0.000	91.21 ± 12.83	0.000
PSQI				
< 5	118.48 ± 17.36		76.60 ± 9.71	
≥ 5	121.26 ± 21.37	0.166	77.73 ± 10.76	0.288
CES–D				
< 16	119.88 ± 19.29		77.24 ± 9.98	
≥ 16	117.90 ± 17.30	0.413	75.98 ± 10.35	0.326
Family history of hypertension				
No	118.27 ± 16.73		76.44 ± 9.09	
Yes	129.64 ± 30.28	0.000	81.78 ± 15.47	0.001

CES-D, Centre for Epidemiologic Studies scale questionnaire; HAART, highly active antiretroviral therapy; PSQI, Pittsburgh Sleep Quality Index

Table 18.2 shows significant direct associations of age, systolic and diastolic blood pressure at the time of HIV infection diagnosis, HRQL (physical), body mass index, and waist circumference with systolic ($P < 0.05$ for each) and diastolic blood pressure ($P < 0.05$ for each) in HIV-infected patients.

Table 18.2: Simple linear regression coefficients with 95% confidence intervals for factors related to systolic and diastolic blood pressure in HIV-infected patients.

	Systolic blood pressure		Diastolic blood pressure	
Factors	**B**	**95% CI**	**β**	**95% CI**
Age	0.37	0.19 to 0.54	0.21	0.12 to 0.30
Physical activity	-0.004	-0.02 to 0.006	-0.005	-0.01 to 0.0002
Body mass index	1.20	0.79 to 1.62	0.70	0.48 to 0.92
Waist circumference	0.44	0.27 to 0.61	0.29	0.20 to 0.38
Blood glucose	0.05	-0.006 to 0.10	0.02	-0.01 to 0.05
PSQI	0.36	-0.27 to 0.99	0.08	-0.26 to 0.42
CESD	-0.12	-0.37 to 0.13	-0.06	-0.19 to 0.08
HRQL (physical)	0.24	0.01 to 0.47	0.12	-0.003 to 0.24
HRQL (mental)	-0.04	-0.25 to 0.16	0.03	-0.08 to 0.14
CD4 cell count	-0.006	-0.01 to 0.002	-0.001	-0.005 to 0.003
HIV duration	-0.03	-0.09 to 0.04	0.02	-0.01 to 0.06
HAART duration	-0.08	-0.16 to 0.002	-0.02	-0.06 to 0.03
Baseline SBP	0.37	0.26 to 0.49	0.17	0.11 to 0.23
Baseline DBP	0.43	0.27 to 0.58	0.20	0.11 to 0.28

β, linear regression coefficient; CESD, Centre for Epidemiologic Studies scale questionnaire; CI, confidence interval; DBP, diastolic blood pressure; HAART, highly active antiretroviral therapy; HRQL, health-related quality of life; PSQI, Pittsburgh Sleep Quality Index; SBP, systolic blood pressure.

18.2 RESULTS OF UNADJUSTED ANALYSES – FACTORS RELATED TO BLOOD PRESSURE CHANGES IN HIV-NEGATIVE INDIVIDUALS.

Tables 18.3 and 18.4 presents the group means (± standard deviation) of systolic and diastolic blood pressure, including the statistical significance of the mean differences, among HIV-negative individuals. Unlike the reported findings among HIV-infected participants, the only variable related to systolic and diastolic blood pressure changes in HIV-negative persons was occupational grade: the mean systolic ($P = 0.021$) and diastolic blood pressure ($P = 0.040$) were statistically significantly higher among middle-income HIV-negative individuals, compared to their counterparts in the working class. Among other variables, age groups and hyperglycaemia were associated with systolic blood pressure changes only: the mean values of systolic blood pressure were significantly higher among HIV-negative individuals aged between 41 and 64 years ($P < 0.001$), and assessed to have hyperglycaemia ($P = 0.027$), compared respectively to their counterparts who were younger, and with blood glucose concentrations within the normal range. Factors associated with changes in diastolic blood pressure alone were alcohol use and sleep quality: HIV-negative individuals who were current drinkers ($P = 0.040$) or assessed to have poor sleep quality ($P = 0.045$) had higher mean values of diastolic blood pressure, compared respectively to HIV-negative individuals who abstained from alcohol or without sleep problems.

Table 18.3: Group means (± standard deviation) of systolic and diastolic blood pressure in HIV-negative individuals.

	Systolic BP (mmHg)		Diastolic BP (mmHg)	
Factors	**Mean ± SD**	***P* value**	**Mean ± SD**	***P* value**
Age group				
18 to 40 years	123.20 ± 13.71		77.51 ± 18.57	
41 to 64 years	136.25 ± 20.73	0.000	81.61 ± 13.25	0.091
Gender				
Female	124.37 ± 15.73		76.97 ± 10.90	
Male	131.01 ± 18.75	0.058	80.78 ± 12.19	0.102
Education				
≤ 12 years	129.56 ± 16.79		78.74 ± 12.91	
> 12 years	126.77 ± 18.16	0.441	79.11 ± 10.97	0.878
Occupational grade				
Middle-income	133.44 ± 17.45		82.27 ± 10.26	
Working class/unemployed	124.92 ± 17.22	0.021	77.32 ± 12.17	0.040
Smoking status				
Never smoker	126.81 ± 17.08		78.41 ± 11.87	
Ever smoker	136.41 ± 20.35	0.090	83.55 ± 9.40	0.171
Drinking status				
Abstainer	126.79 ± 17.71		77.05 ± 10.95	
Drinker	129.48 ± 17.56	0.456	81.91 ± 12.31	0.040
Physical activity				
< 150 minutes/week	127.74 ± 15.72		78.29 ± 10.65	
≥ 150 minutes/week	128.14 ± 21.94	0.918	80.67 ± 14.03	0.356
Body mass index				
< 25 kg/m^2	124.83 ± 17.51		77.05 ± 11.82	
≥ 25 kg/m^2	130.10 ± 17.50	0.138	80.40 ± 11.50	0.156
Central obesity				
No	127.71 ± 19.10		79.45 ± 12.02	
Yes	128.11 ± 14.81	0.913	78.10 ± 11.21	0.579
Hyperglycaemia				
No	127.13 ± 17.36		78.59 ± 11.83	
Yes	141.44 ± 16.62	0.027	86.44 ± 4.55	0.067
Antihypertensive treatment				
No	127.20 ± 17.57		79.10 ± 11.94	
Yes	133.75 ± 17.77	0.267	77.80 ± 9.68	0.741
PSQI				
< 5	126.68 ± 18.92		77.12 ± 12.44	
≥ 5	129.72 ± 15.36	0.401	81.91 ± 9.85	0.045
CESD				
< 16	128.77 ± 18.57		79.79 ± 11.92	
≥ 16	123.29 ± 11.07	0.244	74.94 ± 9.88	0.120
Family history of hypertension				
No	127.39 ± 18.08		79.59 ± 11.52	
Yes	129.21 ± 14.31	0.699	77.56 ± 12.89	0.524

CESD, Centre for Epidemiologic Studies scale questionnaire; PSQI, Pittsburgh Sleep Quality Index

Table 18.4 shows that age, body mass index, and waist circumference were linearly associated with systolic and diastolic blood pressure respectively among the HIV-negative controls. As expected, there was a significant inverse association between health-related quality of life (physical component) and systolic blood pressure [β = - 0.08, 95% CI = - 0.68 to - 0.01)].

Table 18.4: Simple linear regression coefficients with 95% confidence intervals for factors related to systolic and diastolic blood pressure in HIV-infected patients.

	Systolic blood pressure		Diastolic blood pressure	
Factors	**B**	**95% CI**	**β**	**95% CI**
Age	0.53	0.28 to 0.78	0.20	0.03 to 0.38
Physical activity	-0.008	-0.03 to 0.02	0.003	-0.014 to 0.02
Body mass index	0.94	0.25 to 1.62	0.47	0.01 to 0.93
Waist circumference	0.38	0.13 to 0.62	0.19	0.02 to 0.36
Blood glucose	0.06	-0.03 to 0.14	0.04	-0.02 to 0.96
PSQI	0.72	-0.51 to 1.96	0.59	-0.23 to 1.41
CESD	0.01	-0.49 to 0.51	-0.13	-0.46 to 0.20
HRQL (physical)	-0.35	-0.68 to -0.01	-0.03	-0.26 to 0.20
HRQL (mental)	-0.08	-0.47 to 0.32	-0.008	-0.27 to 0.26

β, linear regression coefficient; CI, confidence interval; CESD, Centre for Epidemiologic Studies scale questionnaire; PSQI Pittsburgh Sleep Quality Index.

CHAPTER NINETEEN

RESULTS – ANTIRETROVIRAL TREATMENT STATUS AND HYPERTENSION PREDICTION IN HIV-INFECTED PATIENTS.

In this chapter, I identify the best parsimonious model for predicting hypertension in HIV-infected patients. I also determine whether HAART status improves the predictive value of the model.

19.1 THE IMPACT OF HAART STATUS ON THE PREDICTION OF HYPERTENSION IN HIV-INFECTED PATIENTS

In order to determine whether antiretroviral treatment status improved the clinical prediction of hypertension in HIV-infected patients, simple logistic regression models were first fitted to identify predictors of hypertension that formed the most parsimonious predictive model. Table 19.1 summarizes the data stratified by hypertension status, the unadjusted odds ratios for hypertension and the corresponding R^2. HAART status, age, body mass index, sleep quality (PSQI), drinking status, and family history of hypertension were significantly associated with an increased risk of hypertension ($P < 0.05$ for each); whereas CD4 cell count, HAART duration, and HIV infection duration had no statistically significant impact on hypertension risk.

Table 19.1: Unadjusted odds ratios for hypertension and corresponding fit statistics among HIV-infected patients

	Hypertension			
Characteristics	***Yes*** **(N=60)**	***No*** **(N=340)**	**OR (95% CI)**	**Nagelkerke's R^2**
HAART status				
HAART-naïve*	9 (15.3)	89 (26.3)		
HAART-exposed	50 (84.7)	249 (73.7)	1.99 (0.99 to 4.20)	0.016
Age (years), mean±SD	43.5±10.4	36.9±10.5	1.05 (1.03 to 1.08)	0.077
Gender, *n* (%)				
Female*	35 (58.3)	241 (71.1)		
Male	25 (41.7)	98 (28.9)	1.76 (1.09 to 2.82)	0.016
Smoking status, *n* (%)				
Never smoker*	50 (84.8)	298 (90.3)		
Ever smoker	9 (15.2)	32 (9.7)	1.68 (0.86 to 3.28)	0.007
Drinking status, *n* (%)				
Abstainer*	39 (65.0)	260 (76.7)		
Current drinker	21 (35.0)	79 (23.3)	1.77 (1.08 to 2.90)	0.015
Physical activity (mins/wk), mean±SD	148.8±152.9	171.4±178.0	0.99 (0.97 to 1.01)	0.004
BMI (kg/m^2), mean±SD	26.8±5.4	23.8±4.0	1.15 (1.08 to 1.22)	0.091
Family history of hypertension, *n* (%)				
No*	40 (66.7)	310 (92.5)		
Yes	20 (33.3)	25 (7.5)	6.20 (3.52 to 10.92)	0.111
PSQI, mean±SD	5.0±3.2	4.0±2.9	1.10 (1.01 to 1.20)	0.021
CESD, mean±SD	9.7±8.2	10.0±7.4	1.00 (0.96 to 1.03)	0
HRQL (mental score), mean±SD	51.3±9.0	52.0±9.4	0.99 (0.97 to 1.02)	0.001
HRQL (physical score), mean±SD	49.7±9.1	50.3±7.9	0.99 (0.97 to 1.02)	0.001
CD4 count ($cells/mm^3$), mean±SD	406.3±256.3	462.6±248.9	0.99 (0.98 to 1.00)	0.012
HIV duration (months), mean±SD	41.2±31.3	45.5±28.9	1.00 (0.99 to 1.01)	0.005

N, number of observations; CI, confidence intervals; OR, odds ratio for hypertension; R^2, Nagelkerke's fit statistic (explained variability); BMI, body mass index; PSQI, Pittsburgh Sleep Quality Index; HRQL, health-related quality of life; SD, standard deviation; CESD, Centre for Epidemiology Studies Depression scale; HAART, highly active antiretroviral therapy.
*: reference category in the logistic regression model.

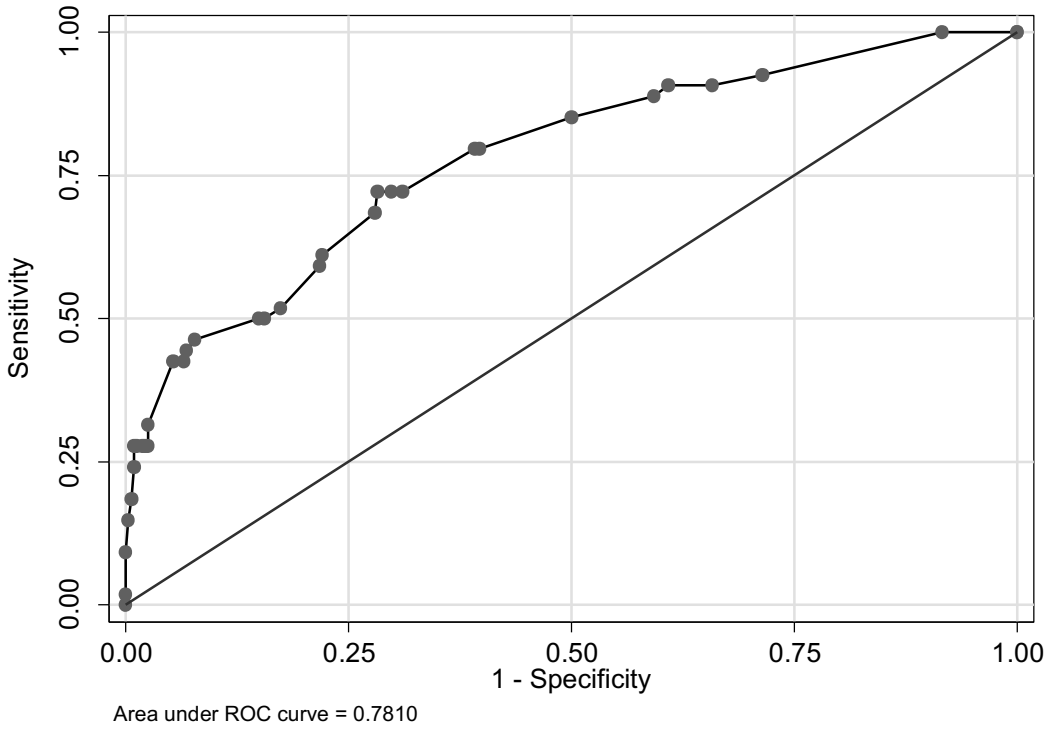
Table 19.2 shows the development of the predictive model and the improvement in degree of fit following the addition of HAART status. Without HAART status, the best predictive model for hypertension in HIV-infected patients comprised the following variables in the order in which they were added: family history of hypertension, body mass index, age, and sleep quality (Nagelkerke's $R^2 = 0.265$) The area under the ROC curve was 0.77, indicating that the predictive model fit moderately well and had good discriminatory ability, in the sense that the model would be able to correctly classify HIV-infected patients by hypertension status 77% of the time. Subsequent addition of HAART status improved the model fit ($R^2 = 0.274$) albeit with only a slight increase in the area under the ROC curve (0.78) (Figure 19.1).

Table 19.2: Developing the best parsimonious model for predicting hypertension using Nagelkerke's R^2

Predictive model	N	Nagelkerke's R^2
Family history of hypertension	395	0.111
+ BMI	389	0.183
+ Age	380	0.244
+ PSQI	374	0.265
+ HAART status	374	0.274

R^2, explained variability (Nagelkerke's fit statistic); N, number of observations; BMI, body mass index; PSQI, Pittsburgh Sleep Quality Index; HAART, highly active antiretroviral therapy.
Model fit (R^2) was improved following addition of HAART status.

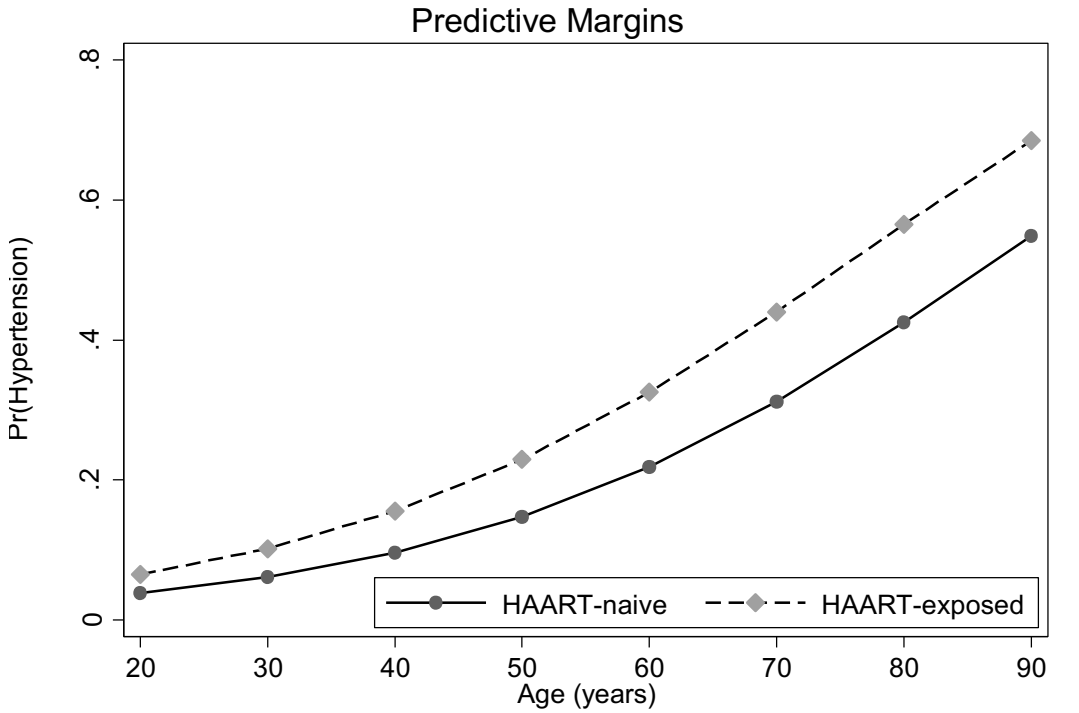
Figure 19.1: Receiver Operating Characteristics (ROC) curve for logistic prediction of hypertension



The model is adjusted for age, body mass index, sleep quality, family history of hypertension, and HAART status

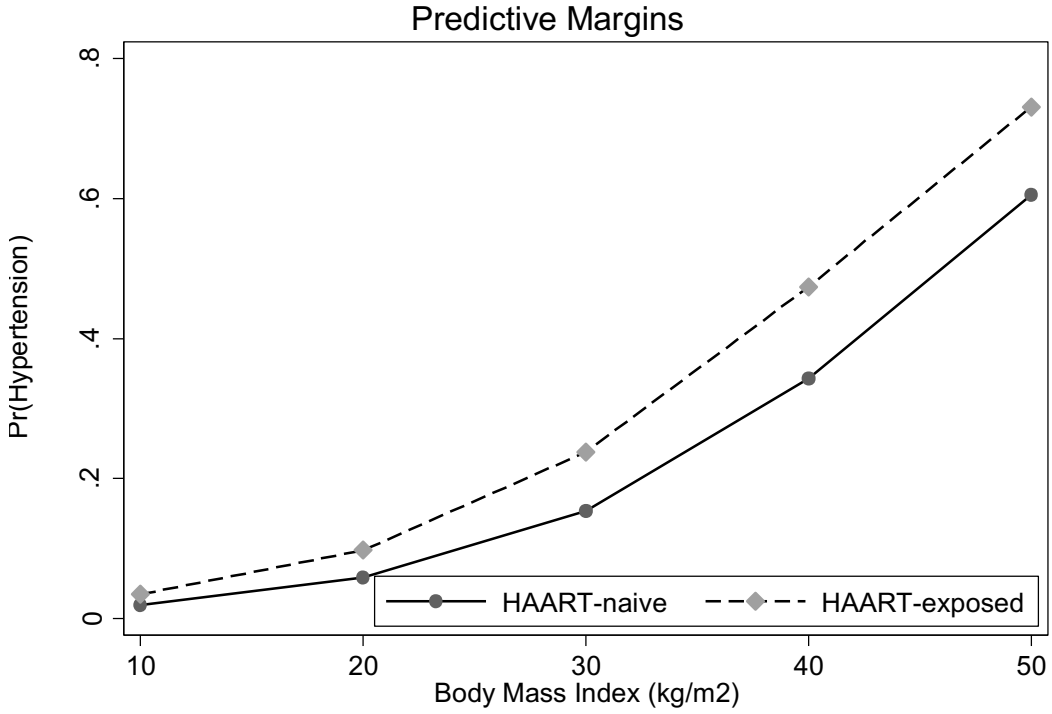
Figures 19.2 to 19.5 show the predictive margins of HAART status at different fixed values of age, body mass index, sleep quality, and family history of hypertension respectively. As illustrated, the predicted probability of hypertension increased with increasing values of each covariate, and was progressively higher for HAART-exposed patients compared to HAART-naïve patients for every additional unit increase in the value of each covariate.

Figure 19.2: Predicted probability of hypertension by age and HAART status



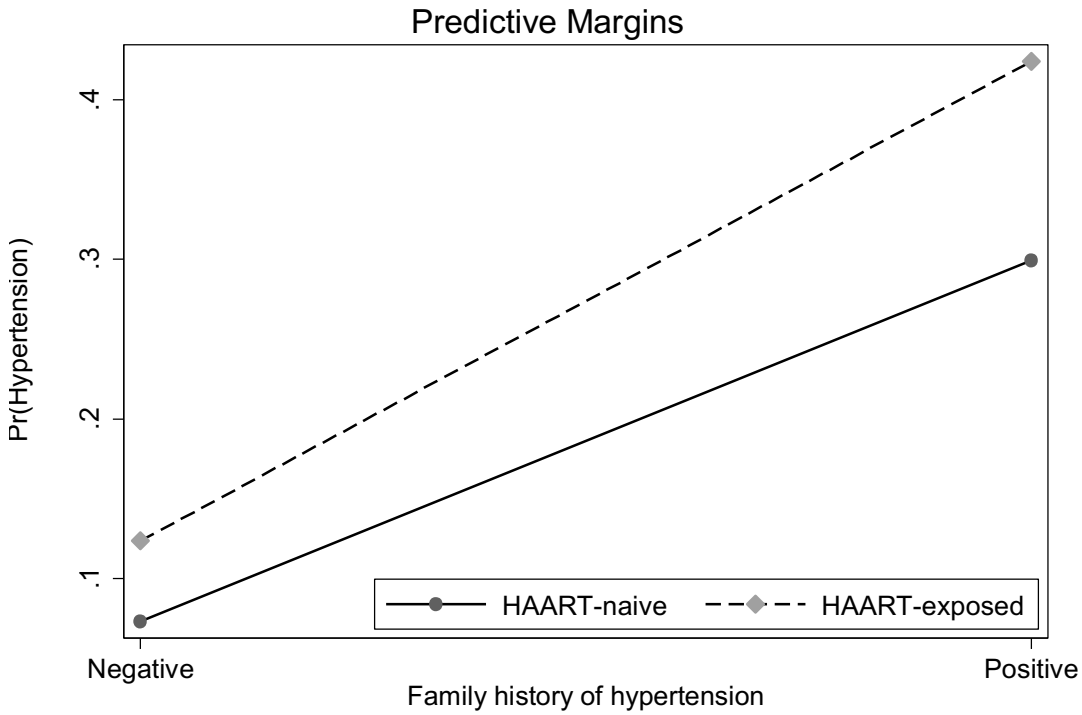
HAART, highly active antiretroviral therapy

Figure 19.3: Predicted probability of hypertension by body mass index and HAART status



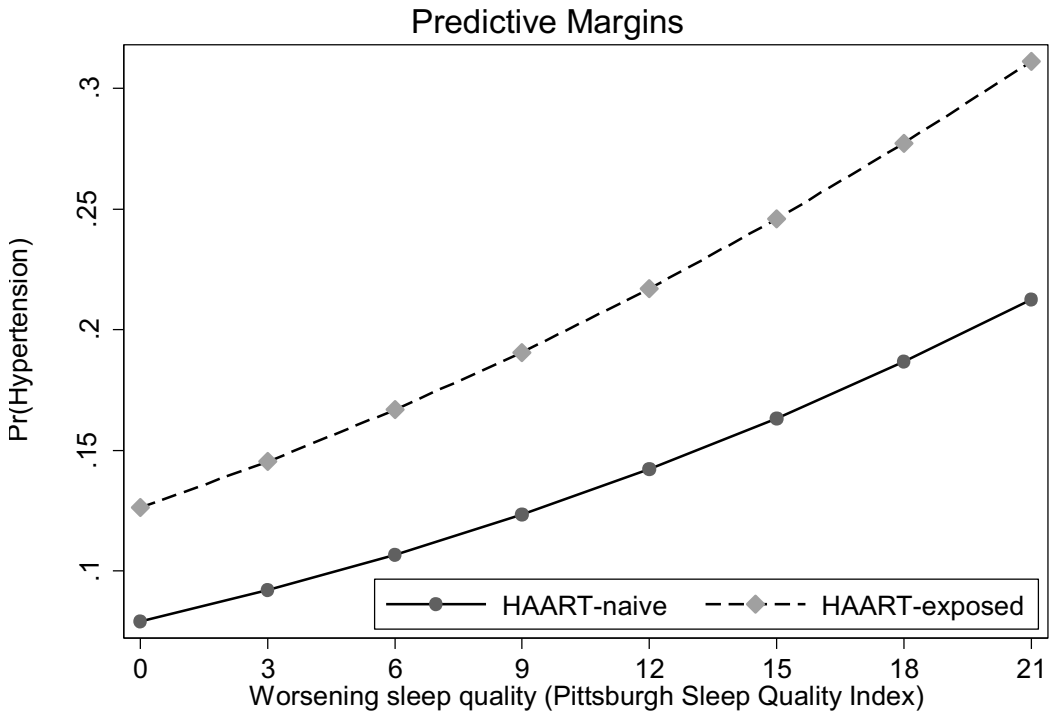
HAART, highly active antiretroviral therapy

Figure 19.4: Predicted probability of hypertension by family history and HAART status



HAART, highly active antiretroviral therapy

Figure 19.5: Predicted probability of hypertension by sleep quality and HAART status



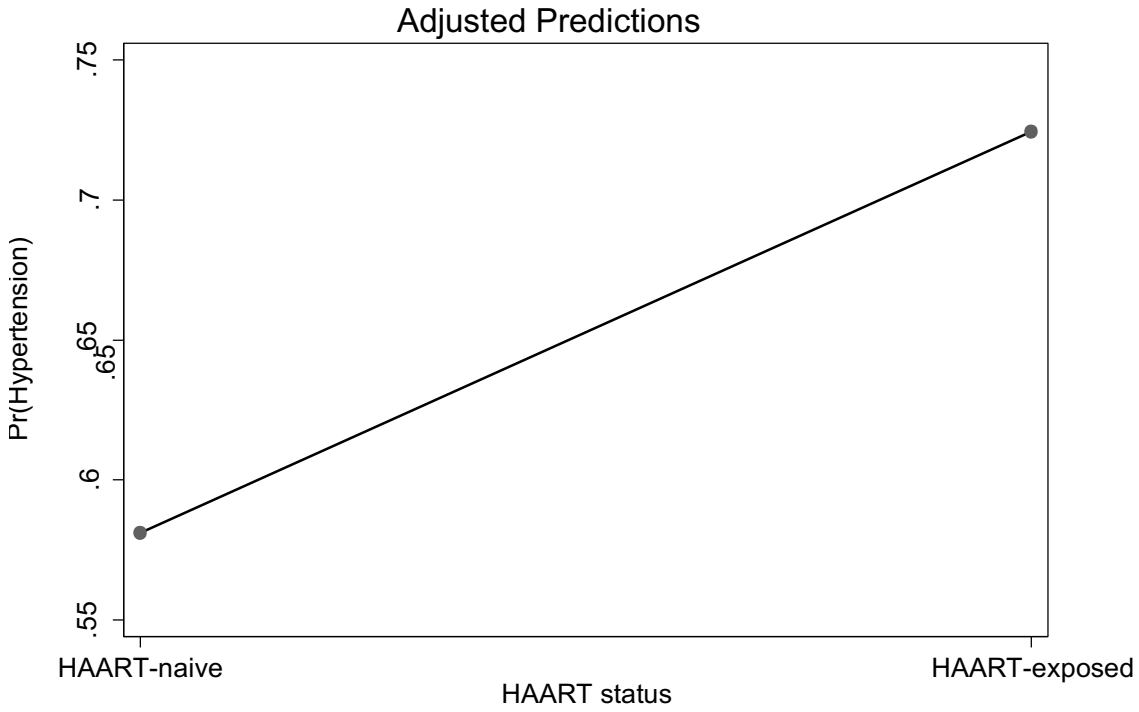
HAART, highly active antiretroviral therapy

Taking into account all variables in the predictive model, the predicted probability of hypertension was 46.9% for an average HAART-naïve patient and 48.5% for a HAART-exposed patient.

Figure 19.6 illustrates a practical application the model using predictive margins. As shown, the predicted probability of hypertension in a hypothetical 50 year old HAART-naïve HIV-infected patient who is overweight (BMI = 26kg/m^2), assessed to have poor sleep quality (PSQI = 10), and a positive family history of hypertension is estimated to be 0.58 (95% CI 0.30 to 0.86). Conversely, if the same patient were to be HAART-exposed,

the predicted probability of hypertension would be increased by approximately 26%: (0.73, 95% CI 0.54 to 0.91).

Figure 19.6: Covariate-adjusted probability of hypertension by HAART status



Results of a practical application of the predictive model showing the predicted probability of hypertension in a hypothetical 50-year-old HAART-naïve HIV-infected patient who is overweight (BMI = 26kg/m^2), assessed to have poor sleep quality (PSQI = 10) and a positive family history of hypertension, as well as a hypothetical HAART-exposed patient with similar characteristics. HAART, highly active antiretroviral therapy.

CHAPTER TWENTY

RESULTS – CAUSAL MEDIATION ANALYSIS

In this chapter, I identify alternative mediating mechanisms of the association between antiretroviral therapy and increased blood pressure.

20.1 RESULTS OF CAUSAL MEDIATION ANALYSES

As previously discussed, the mediation effect is essentially the co-efficient of the indirect effect, which was computed using SEM in Stata. Table 20.1 summarises the indirect effects of HAART on systolic and diastolic blood pressure that were mediated by body mass index, waist circumference, blood glucose, and quality of sleep, as well as the amounts of the total effects of HAART on systolic and diastolic blood pressure that were mediated through each proposed mediator.

Table 20.1: Total, direct and indirect effects of antiretroviral therapy on systolic and diastolic blood pressure

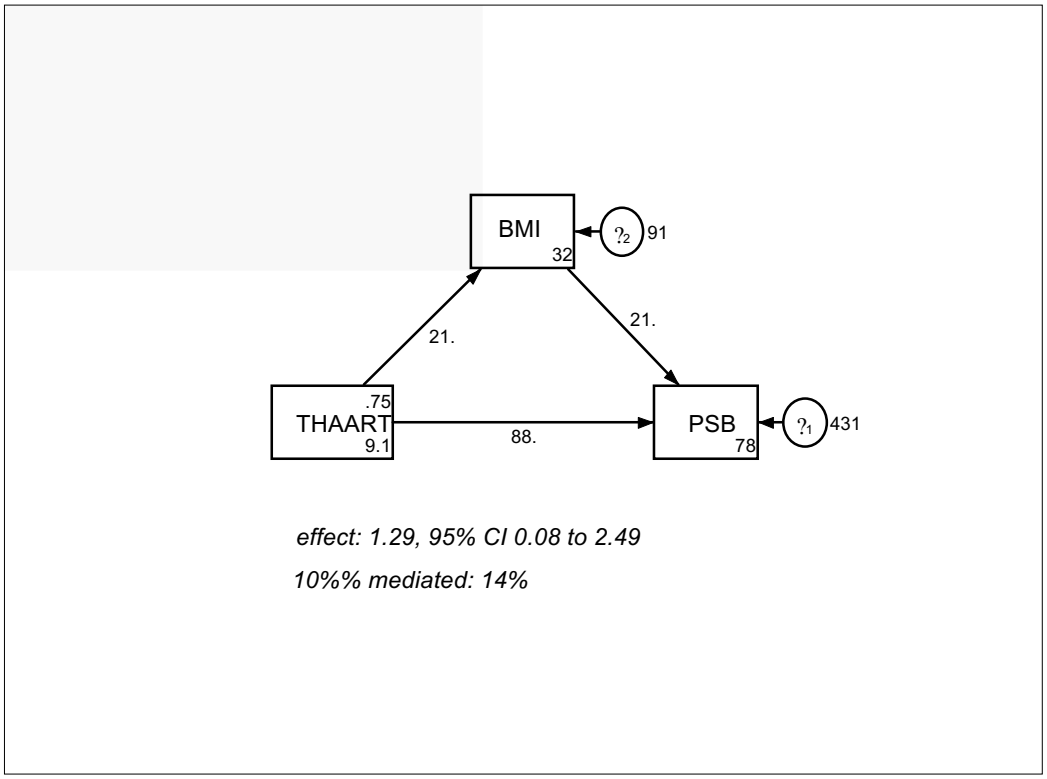
Causal variable	Mediator Variable	Dependent variable	Path A Coef. (95% CI)	Path B Coef. (95% CI)	Total effect Coef. (95% CI)	Direct effect Coef. (95% CI)	Indirect effect Coef. (95% CI)	% mediated
	Body mass index	Systolic BP	1.16 (0.16 to 2.16)	1.11 (0.70 to 1.52)	9.57 (5.32 to 13.82)	8.28 (4.15 to 12.41)	1.29 (0.08 to 2.49)	14
HAART		Diastolic BP	1.16 (0.16 to 2.16)	0.62 (0.42 to 0.83)	7.49 (5.30 to 9.69)	6.77 (4.65 to 8.88)	0.73 (0.06 to 1.40)	10
	Waist	Systolic BP	3.42 (0.92 to 5.92)	0.39 (0.23 to 0.56)	9.34 (5.01 to 13.32)	7.96 (3.86 to 12.06)	1.38 (0.24 to 2.52)	15
HAART	circumference	Diastolic BP	3.42 (0.92 to 5.92)	0.26 (0.17 to 0.34)	7.59 (5.35 to 9.67)	6.71 (4.60 to 8.81)	0.88 (0.19 to 1.58)	12
	Blood glucose	Systolic BP	4.94 (-3.06 to 12.94)	0.04 (-0.01 to 0.09)	8.95 (4.78 to 13.11)	8.74 (4.58 to 12.91)	0.21 (-0.21 to 0.62)	2
HAART	level	Diastolic BP	4.94 (-3.06 to 12.94)	0.01 (-0.02 to 0.04)	7.15 (4.99 to 9.29)	7.09 (4.93 to 9.24)	0.06 (-0.11 to 0.22)	1
	Sleep quality	Systolic BP	-0.02 (-0.71 to 0.67)	0.36 (-0.25 to 0.97)	8.84 (4.63 to 13.04)	8.84 (4.63 to 13.04)	0.002 (-0.23 to 0.24)	0
HAART	(PSQI)	Diastolic	-0.02 (-0.71 to 0.67)	0.08 (-0.23 to 0.40)	7.19 (5.00 to 9.37)	7.19 (5.00 to 9.37)	0.0005 (-0.05 to 0.06)	0

Abbreviations: CI, confidence interval; HAART, highly active antiretroviral therapy.
Path A – regression coefficient for the causal variable when the mediator variable is regressed on the causal variable.
Path B – regression coefficient for the mediator variable when the dependent variable is regressed on the mediator variable, adjusted for the causal variable.
Direct effect – the effect of the causal variable on the dependent variable adjusted for the mediator variable.
Total effect – the effect of the causal variable on the dependent variable without adjusting for the mediator variable.
Indirect effect – the indirect effect of the causal variable on the dependent variable through the mediator variable.
% mediated – the amount (in percentage) of the total effect is attributed to the mediator variable.
95% confidence intervals for indirect effects are bias-corrected.

20.2 THE EFFECT OF HAART ON BLOOD PRESSURE MEDIATED BY BODY MASS INDEX

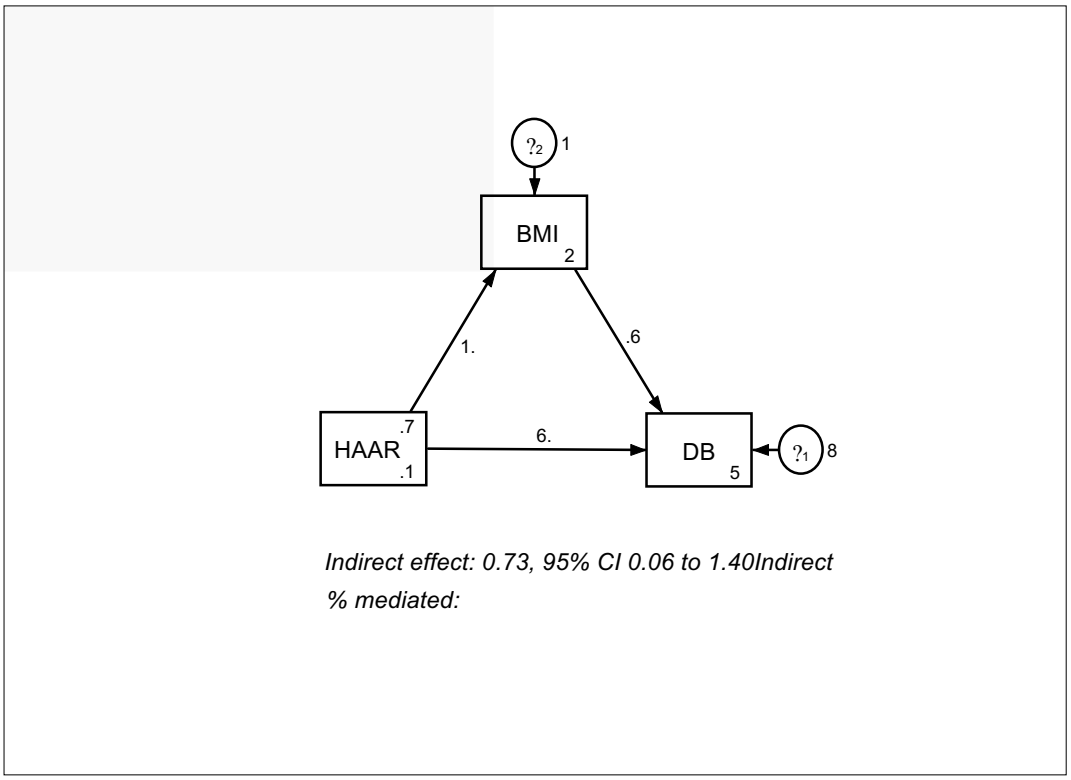
The indirect effect of HAART on systolic blood pressure that was mediated by body mass index was statistically significant (coefficient = 1.29, 95% CI 0.08 to 2.49), and accounted for 14% of the total effect of HAART exposure on systolic blood pressure. The mediation analysis also showed a statistically significant indirect effect of HAART on diastolic blood pressure through body mass index (coefficient = 0.73, 95% CI 0.06 to 1.40), accounting for 10% of the total effect of HAART on diastolic blood pressure (see path diagrams in Figures 20.1 and 20.2).

Figure 20.1: Path diagram of a mediational model showing the indirect effect of antiretroviral therapy on systolic blood pressure through body mass index



BMI, body mass index; CI, confidence interval; HAART, highly active antiretroviral therapy; SBP, systolic blood pressure

Figure 20.2: Path diagram of a mediational model showing the indirect effect of antiretroviral therapy on diastolic blood pressure through body mass index

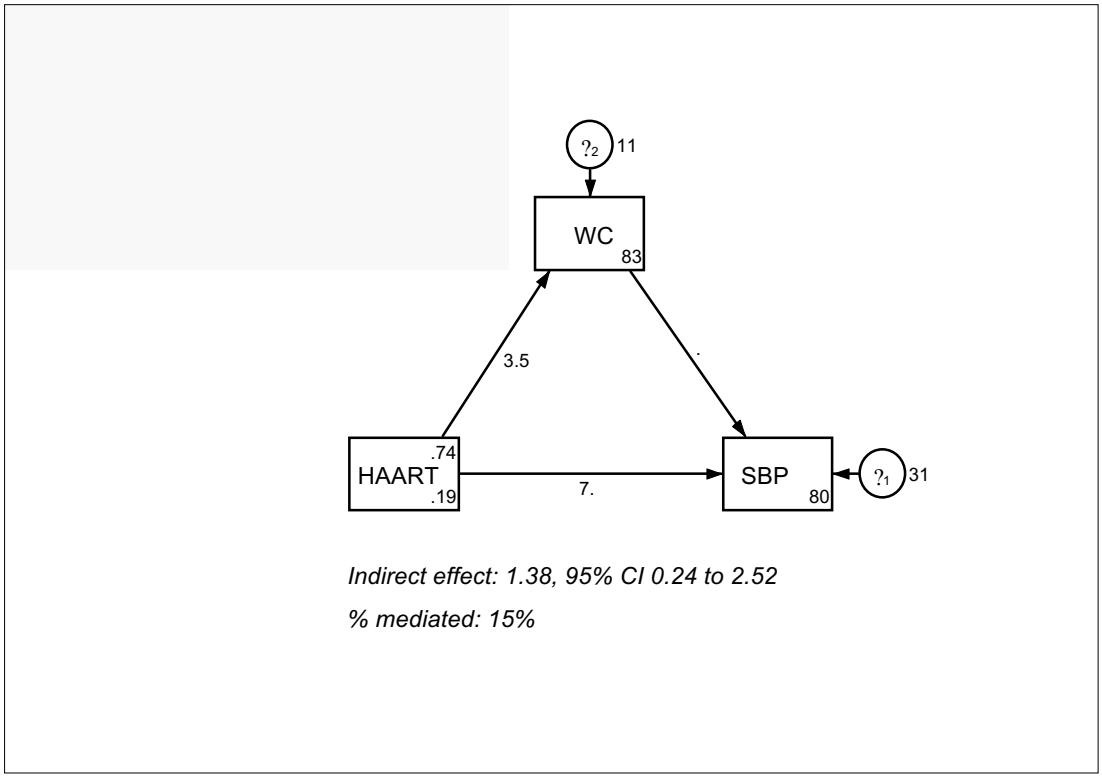


BMI, body mass index; CI, confidence interval; HAART, highly active antiretroviral therapy; DBP, diastolic blood pressure

20.3 THE EFFECT OF HAART ON BLOOD PRESSURE MEDIATED BY WAIST CIRCUMFERENCE

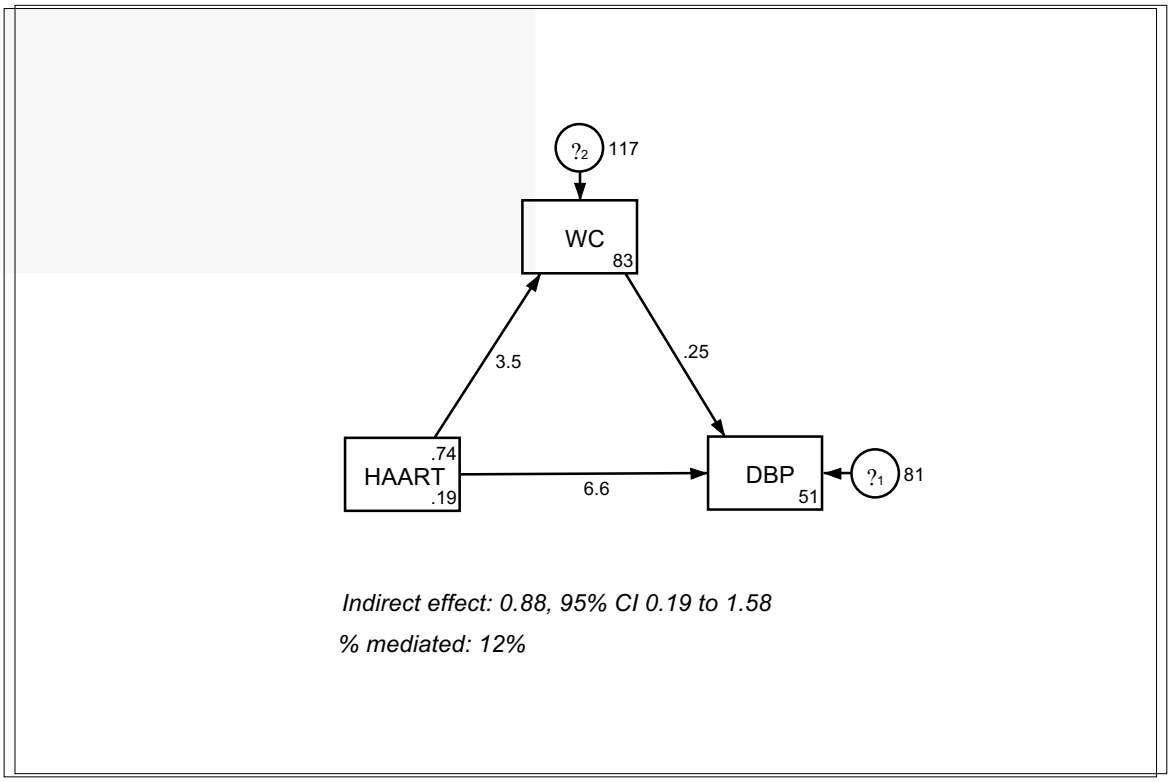
The indirect effect of HAART on systolic blood pressure that was mediated by waist circumference was statistically significant (coefficient = 1.38, 95% CI 0.24 to 2.52), mediating 15% of the total effect of HAART exposure on systolic blood pressure. Similarly, waist circumference was a statistically significant partial mediator of the effect of HAART exposure on diastolic blood pressure (coefficient = 0.88, 95% CI 0.19 to 1.58), accounting for 12% of the total effect of HAART on diastolic blood pressure (see path diagrams in Figures 20.3 and 20.4).

Figure 20.3: Path diagram of a mediational model showing the indirect effect of antiretroviral therapy on systolic blood pressure through waist circumference



CI, confidence interval; HAART, highly active antiretroviral therapy; SBP, systolic blood pressure; WC, waist circumference.

Figure 20.4: Path diagram of a mediational model showing the indirect effect of antiretroviral therapy on diastolic blood pressure through waist circumference



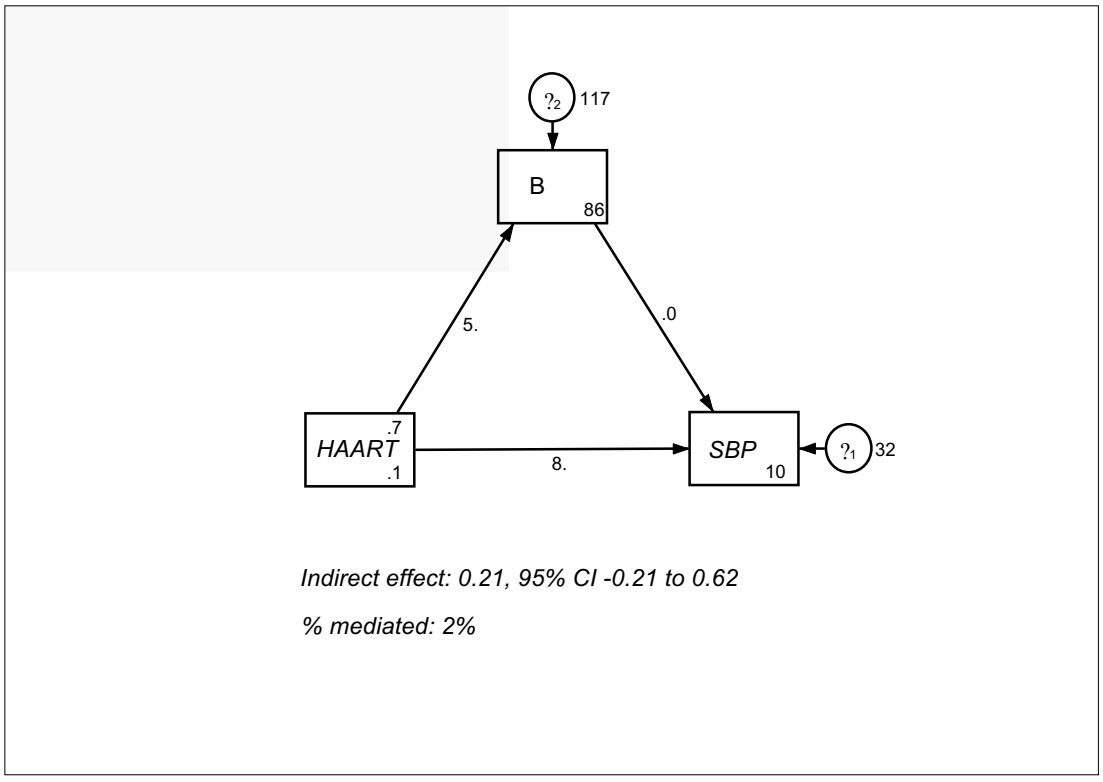
CI, confidence interval; HAART, highly active antiretroviral therapy; DBP, diastolic blood pressure; WC, waist circumference.

20.4 THE EFFECT OF HAART ON BLOOD PRESSURE MEDIATED BY BLOOD GLUCOSE LEVEL

Blood glucose concentration was no significant mediator of the effect of HAART on systolic (coefficient = 0.21, 95% CI -0.21 to 0.62) or diastolic blood pressure (coefficient

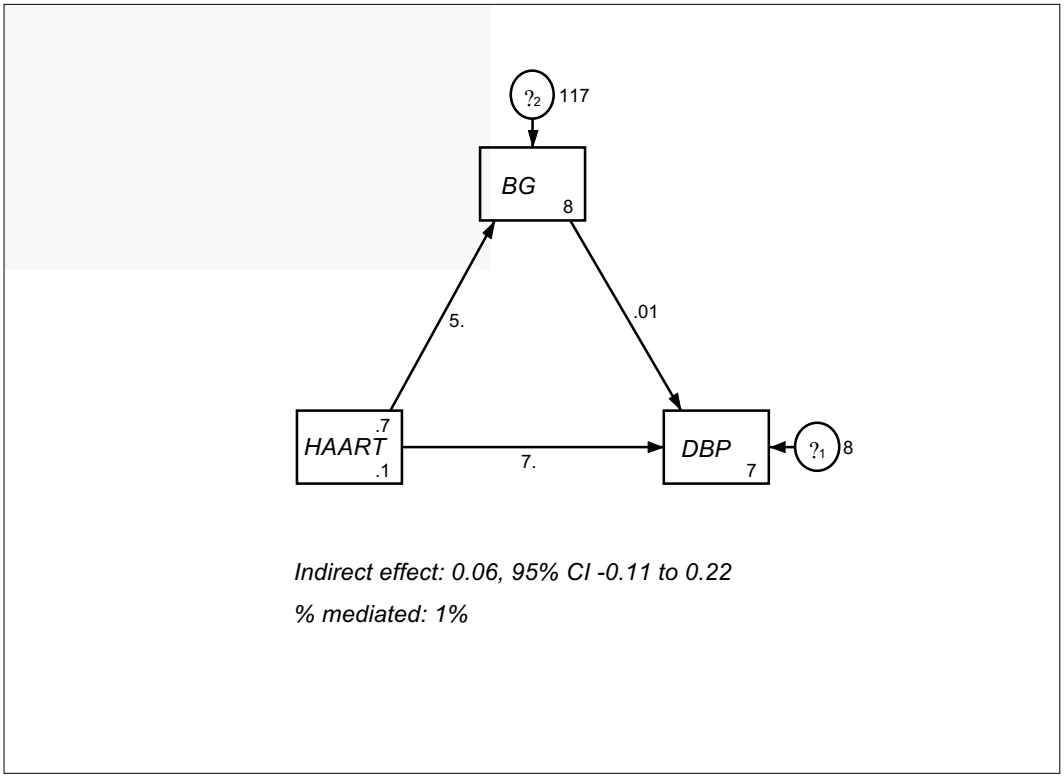
= 0.06, 95% CI -0.11 to 0.21), accounting only for 2% and 1% of the total effects of HAART on systolic and diastolic blood pressure respectively (see path diagrams in Figures 20.5 and 20.6).

Figure 20.5: Path diagram of a mediation model showing the indirect effect of antiretroviral therapy on systolic blood pressure through blood glucose level



CI, confidence interval; HAART, highly active antiretroviral therapy; SBP, systolic blood pressure; BG, blood glucose

Figure 20.6: Path diagram of a mediational model showing indirect effect of antiretroviral therapy on diastolic blood pressure through blood glucose level

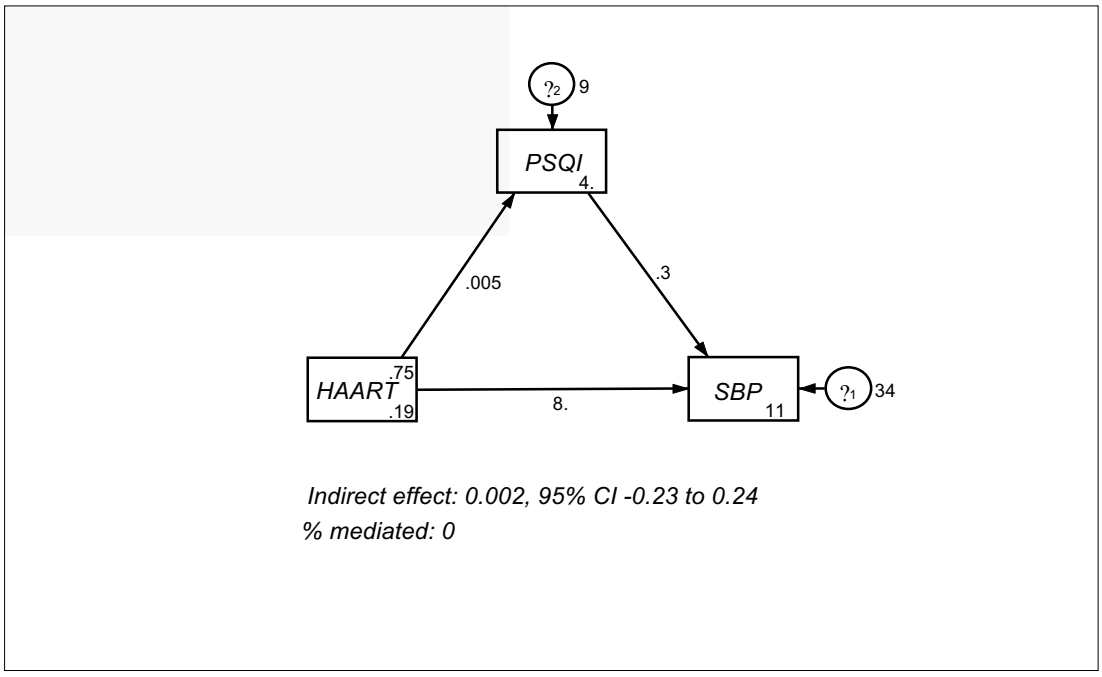


CI, confidence interval; HAART, highly active antiretroviral therapy; DBP, diastolic blood pressure; BG, blood glucose

20.5 THE EFFECT OF HAART ON BLOOD PRESSURE MEDIATED BY SLEEP QUALITY

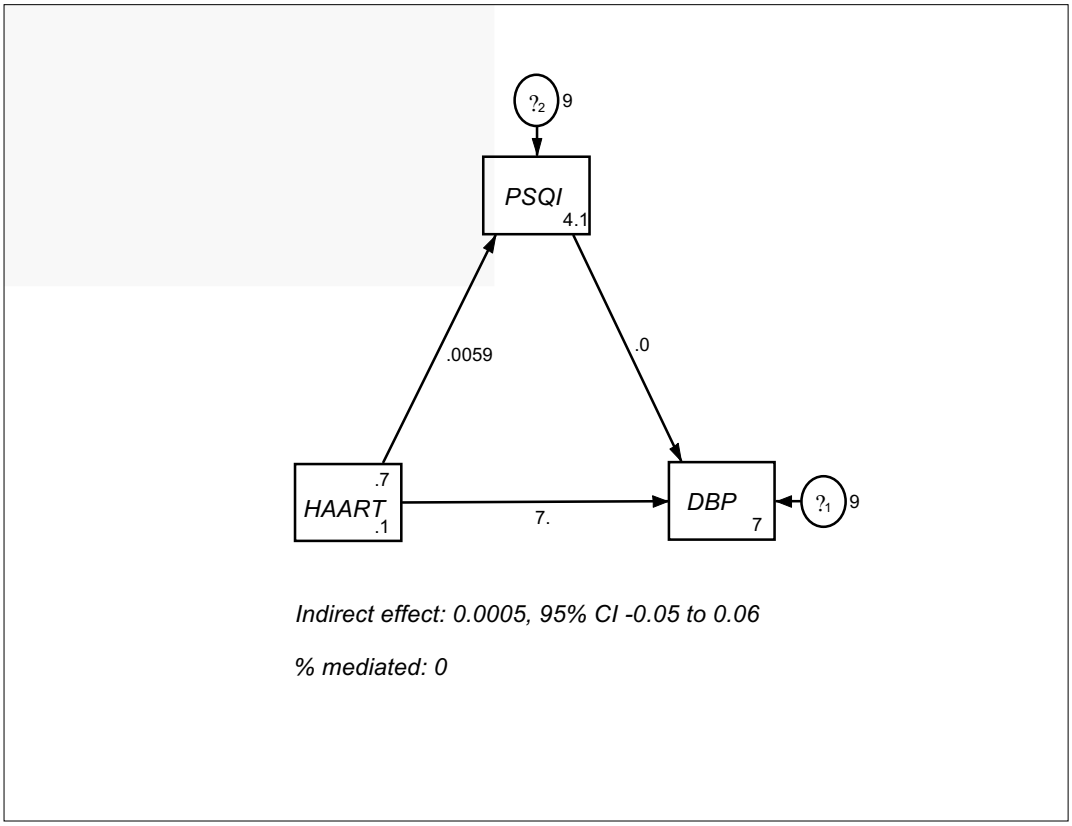
The quality of sleep, as measured by the PSQI, was no mediator of the effect of HAART on systolic (coefficient = 0.002, 95% CI -0.23 to 0.24) or diastolic blood pressure (coefficient = 0.0005, 95% CI -0.05 to 0.06). The direct and total effects of HAART on systolic and blood pressure were equal, so that the indirect effects through sleep quality were non-existent (see path diagrams in Figures 20.7 and 20.8).

Figure 20.7: Path diagram of a mediational model showing the indirect of antiretroviral therapy on systolic blood pressure through sleep quality



CI, confidence interval; HAART, highly active antiretroviral therapy; SBP, systolic blood pressure; PSQI, Pittsburgh Sleep Quality Index

Figure 20.8: Path diagram of a mediation model showing the indirect effect of antiretroviral therapy on diastolic blood pressure through sleep quality



CI, confidence interval; HAART, highly active antiretroviral therapy; DBP, diastolic blood pressure; PSQI, Pittsburgh Sleep Quality Index

Although blood glucose level and sleep quality were not mediators of the effects of antiretroviral therapy on systolic and diastolic blood pressure, however it is important to mention that sleep quality was a strong moderator of these effects. As shown in Table 20.2, the effects of antiretroviral therapy on systolic and diastolic blood pressure remained significantly greater in HIV-infected patients with poor sleep quality (PSQI ≥ 5), compared to those reporting a good sleep quality (PSQI < 5), even after controlling for potential confounders ($P < 0.001$ for all).

Table 20.2: Sleep quality as a moderator of the effects of antiretroviral therapy on systolic and diastolic blood pressure

	PSQI < 5		**PSQI ≥5**		**PSQI < 5 *vs* PSQI ≥5**	
	SBP	**DBP**	**SBP**	**DBP**	**SBP**	**DBP**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**(*P* value)**	**(*P* value)**
HAART	6.08 (1.19 to 10.98)	5.99 (3.31 to 8.68)	14.34 (6.23 to 22.44)	9.60 (5.67 to 13.54)	$P < 0.001$	$P < 0.001$
HAART*	5.03 (0.04 to 10.02)	5.49 (2.76 to 8.22)	13.44 (5.44 to 21.43)	9.21 (5.34 to 13.07)	$P < 0.001$	$P < 0.001$
HAART†	4.86 (-0.03 to 9.74)	5.43 (2.79 to 8.07)	11.10 (2.94 to 19.25)	8.25 (4.36 to 12.14)	$P < 0.001$	$P < 0.001$
HAART‡	4.18 (-0.90 to 9.26)	5.06 (2.32 to 7.79)	12.85 (4.46 to 21.24)	8.54 (4.55 to 12.52)	$P < 0.001$	$P < 0.001$
HAART§	5.32 (-0.30 to 10.94)	5.93 (2.86 to 9.01)	14.80 (4.46 to 25.14)	8.60 (3.92 to 13.38)	$P < 0.001$	$P < 0.001$

*, models adjusted age and sex;
†, models adjusted for age, sex and body mass index;
‡, models adjusted for age, sex, body mass index, smoking status, drinking status and physical activity;
§, models adjusted for age, sex, body mass index, smoking status, drinking status, physical activity, CD4 cell count and HIV infection duration.
β, regression coefficient; CI, confidence interval; DBP, diastolic blood pressure; HAART, highly active antiretroviral therapy; PSQI, Pittsburgh Sleep Quality Index; SBP, systolic blood pressure.

20.6 THE CAUSAL INFERENCE APPROACH TO MEDIATION

To reiterate, the causal inference approach entailed fitting the mediation models while controlling for omitted variables (or potential confounders) and ruling out alternative models. Given that body mass index and waist circumference were significant mediators of the effects of HAART on systolic and diastolic blood pressure, results of the covariate-adjusted mediation effects of body mass index and waist circumference alone are presented in Table 20.3.

Table 20.3: Covariate-adjusted indirect effects of body fat measures on the associations of antiretroviral therapy with systolic and diastolic blood pressure

Causal Variable	Mediator variable	Outcome variable	Indirect effect* Coef. (95% CI)	% med*	Indirect† Coef. (95% CI)	% med†	Indirect effect‡ Coef. (95% CI)	% med‡
HAART	Body mass index	Systolic BP	1.32 (0.48 to 2.16)	14	1.36 (0.13 to 2.60)	14	0.98 (-0.44 to 2.40)[NS]	10
		Diastolic BP	0.74 (0.05 to 1.43)	10	0.76 (0.08 to 1.44)	10	0.52 (-0.23 to 1.27)[NS]	7
HAART	Waist circumference	Systolic BP	1.18 (0.15 to 2.21)	13	1.05 (0.07 to 2.04)	11	0.94 (0.73 to 2.43)	10
		Diastolic BP	0.78 (0.14 to 1.41)	10	0.70 (0.09 to 1.30)	9	0.64 (0.16 to 2.09)	9

***** – indirect effect controlled for age (continuous) and sex;

† – indirect effect controlled for age (continuous), sex, smoking status, drinking status, and physical activity (continuous);

‡ – indirect effect controlled for age (continuous), sex, smoking status, drinking status, physical activity (continuous), CD4 cell count and HIV infection duration

% med***** – proportion of the total effect of HAART on each outcome variable that is attributed indirectly to the mediator variable, adjusted for age and sex.

% med† – proportion of the total effect of HAART on each outcome variable that is attributed indirectly to the mediator variable, adjusted for age, sex, smoking status, drinking status, and physical activity (continuous).

% med‡ – proportion of the total effect of the independent variable on the dependent variable that is attributed indirectly to the mediator variable, adjusted for age, sex, smoking status, drinking status, physical activity (continuous), CD4 cell count and HIV infection duration.

NS – not statistically significant at 5% level; 95% confidence intervals for indirect effects are bias-corrected.

CI, confidence interval; HAART, highly active antiretroviral therapy

20.6.1 Covariate-adjusted indirect effects of HAART exposure on blood pressure through waist circumference

After controlling for age and sex, waist circumference remained a significant partial mediator of the effects of HAART exposure on systolic blood pressure (coefficient = 1.18, 95% CI 0.15 to 2.21) and diastolic blood pressure (coefficient = 0.78, 95% CI 0.14 to 1.41), accounting for 13% and 10% of the total effects of HAART on systolic and diastolic blood pressure respectively. Additional adjustments for lifestyle factors, including smoking status, drinking status, and physical activity levels, revealed that waist circumference accounted for 11% (coefficient = 1.05, 95% CI 0.07 to 2.04) and 9% (coefficient = 0.70, 95% CI 0.09 to 1.30) of the total effects of HAART on systolic and diastolic blood pressure respectively. Unlike body mass index, waist circumference still remained a statistically significant partial mediator of the total effects of HAART on systolic blood pressure (coefficient 0.94, 95% CI 0.73 to 2.43) and diastolic blood pressure (coefficient 0.64, 95% CI 0.16 to 2.09) even after further adjustments for duration of HIV infection and CD4 cell count, accounting for 10% and 9% of the respective total effects.

20.6.2 Ruling out the reverse causal model

Reverse causation was ruled out theoretically as a temporal relationship where blood pressure changes lead to increased body mass index or waist circumference is biologically implausible. Similarly, neither blood pressure nor body fat changes are indications for HAART; it is impossible for these biological changes to influence HAART exposure in HIV-infected patients. Kenny (2015) asserts that it is scientifically valid to rule out reverse causality theoretically.

20.6.3 Ruling out HAART status as a potential effect-modifier

To reiterate, any alternative model that may explain the interaction between HAART, body fat measures, and blood pressure changes in HIV-infected patients could potentially invalidate any causal inferences about the mediation models. Hence, any such model had to be ruled out. As shown in Table 20.4, HAART status was not a moderator of the association between body fat changes and blood pressure changes. Body mass index was directly associated with systolic and diastolic blood pressure in HAART-exposed ($P < 0.05$ for each) and HAART-naïve patients ($P < 0.05$ for each), and likelihood ratio tests revealed no statistically significant difference between models ($P = 1.000$ for all). Similarly, waist circumference was directly associated with systolic and diastolic blood pressure in HAART-exposed ($P < 0.05$ for each) and HAART-naïve patients ($P < 0.05$ for each), with no statistically significant difference between models as revealed by likelihood ratio tests ($P = 1.000$ for all).

Table 20.4: Linear regression analyses of blood pressure on body fat measures stratified by antiretroviral treatment status.

	HAART-exposed		**HAART-naïve**		**HAART-exposed *vs* HAART-naive**	
	Systolic BP β (95% CI)	**Diastolic BP β (95% CI)**	**Systolic BP β (95% CI)**	**Diastolic BP β (95% CI)**	**Systolic BP (*P*-value)**	**Diastolic BP (*P*-value)**
BMI	1.13 (0.50 to 1.75)	0.88 (0.52 to 1.23)	1.10 (0.60 to 1.61)	0.55 (0.29 to 0.80)	*P* = 1.000	*P* = 1.000
WC	0.48 (0.25 to 0.70)	0.31 (0.18 to 0.44)	0.36 (0.15 to 0.58)	0.23 (0.12 to 0.34)	*P* = 1.000	*P* = 1.000

BMI, body mass index; BP, blood pressure; HAART, highly active antiretroviral therapy; WC, waist circumference.

CHAPTER TWENTY-ONE

RESULTS – PROPENSITY SCORE MATCHING

In this chapter, I estimate the causal average treatment effect of antiretroviral therapy on blood pressure.

21.1 DESCRIPTION OF THE ESTIMATED PROPENSITY SCORE

In order to estimate the average treatment effect for the treated (ATT) of HAART on blood pressure, the propensity score analysis was restricted to matching only HAART-exposed patients with HAART-naïve patients. The region of common support obtained by fitting the propensity score model was (0.1857, 0.9995), so that the estimated propensity scores fell within these bounds (inclusive). The mean (± standard deviation) propensity score (0.7642 ± 0.1994) was less than the median (0.8308 [interquartile range: 0.6240, 0.9299]), which may suggest that the distribution of the propensity scores was skewed to the left. However, as determined by the Skewness value (-0.777), the extent to which the distribution of the propensity scores deviated from symmetry around the mean remained acceptable within the bounds of a normally distributed data; in fact, as a rule of thumb, Skewness values that range from -1 to +1 are considered good enough to prove a normal distribution (George & Mallery, 2010). Nonetheless, the propensity scores were transformed to a logarithmic scale as a measure for dealing with any potential consequences of non-normality such as type I or type II errors (Osborne, 2002).

Propensity scores were grouped into five optimal blocks, so that the mean propensity score in each subgroup or block was no different between HAART-exposed and HAART-naïve participants. In addition, the characteristics on which the propensity scores were

subsequently matched were also similar between patients naïve and exposed to HAART in all five blocks. These indicated that the balancing property of the propensity score was satisfied.

21.2 MATCHING ON THE PROPENSITY SCORE

The standard deviation of the logit of the propensity score was 1.6121. The propensity score matching entailed using nearest neighbour matching with a specified calibre width of 0.2, so that 0.2 of the standard deviation of the logit of the propensity score was 0.3224. Therefore, the logits of the propensity score differed by no more than 0.3224 between matched treated (HAART-exposed) and untreated (HAART-naïve) HIV-infected patients.

As mentioned, there were 306 HAART-exposed and 100 HAART-naïve HIV-infected participants before propensity score matching was performed. However, matching these patients in both groups on the logit of the propensity score yielded 229 HAART-treated participants and 74 HAART-naïve participants. However, there still remained baseline covariates that were statistically significantly different between HAART-exposed and naïve patients after fitting this initial propensity score matching model: occupational grade, educational attainment, physical activity levels, baseline diastolic blood pressure, health-related quality of life scores on the mental scale, and depression status ($P < 0.05$ for each). The standardised bias after propensity score matching was 15.4%, and the variance ratios were high for three continuous variables (variance ratio ranging from 0.76 to 1.32), alluding further to the unbalanced distribution of covariates between HAART-exposed and HAART-naïve patients. Table 21.1 describes the characteristics of HAART-exposed and HAART-naïve patients in this initial propensity score matched sample.

Eliminating the residual differences in the baseline characteristics entailed additional modifications to the estimated propensity score model and repeating the matching process. The propensity score model was modified by adding interaction terms at random (Caliendo & Kopeining, 2006; Dehejia & Wahba, 1999), and re-assessed for similarity in the baseline covariates between HAART-exposed and HAART-naïve participants: adding the interaction of blood glucose concentration and body mass index to the initial propensity score model resulted in a propensity score matched sample, still comprising 229 HAART-exposed patients and 74 HAART-naïve patients, but without any observed systematic difference in the baseline characteristics between HAART-exposed and HAART-naïve patients. The means and proportions of all baseline covariates were not significantly different between HAART-exposed and HAART-naïve patients in this matched sample; the standardised bias after matching was 5%; and the largest variance ratio was 1.66 (variance ratio ranging from 0.59 to 1.70). Hence, the distribution of baseline characteristics between the treatment groups was balanced. Table 21.2 describes the characteristics of the HAART-exposed and HAART-naïve participants in this second and final propensity score matched sample.

Table 21.1: Characteristics of HAART-exposed and HAART-naïve patients in the first propensity score matched sample

Variable	HAART-exposed (N = 229)	HAART-naïve (N = 74)	*P* value	% bias	VR
	Means / Proportions				
Age (years)	37.48	39.26	0.216	-15.4	0.29
Males	29.95 %	29.95 %	1.000	0	–
Education > 12 years	42.64 %	31.47 %	0.022 *	25.0	–
Working class / unemployed	73.60 %	84.77 %	0.006 *	-28.9	–
Ever smoker	10.15 %	7.11 %	0.283	10.8	–
Current drinker	26.40 %	22.84 %	0.414	8.2	–
Physical activity (minutes/week)	156.28	118.98	0.026 *	20.6	1.10
Body mass index (kg/m^2)	24.18	24.92	0.109	-16.16	0.71
Waist circumference (cm)	85.73	88.78	0.080	-25.6	0.23
Blood glucose (mmol/L)	4.91	4.97	0.717	-4.5	0.32
On antihypertensive treatment	1.52 %	0	0.082	10.8	–
PSQI ≥ 5	33.00 %	24.37 %	0.058	18.1	–
CESD ≥ 16	18.78 %	8.12 %	0.002 *	26.9	–
HRQL (physical)	50.69	50.72	0.962	-0.5	1.29
HRQL (mental)	51.74	54.68	0.001	-30.3	1.68†
Family history of hypertension	10.15 %	5.58 %	0.093	13.8	–
CD4 cell count (cells/mm^3)	444.30	446.92	0.912	-1.1	1.14
HIV infection duration (months)	45.41	46.21	0.767	-3.1	1.31
Baseline SBP (mmHg)	112.10	115.43	0.059	-19.2	0.75
Baseline DBP (mmHg)	74.36	77.92	0.002 *	-29.2	1.35†

–, not applicable to proportions; *, $P < 0.05$; †, variance ratio is greater than upper limit of 1.32 set by Stata; CESD, Centre for Epidemiologic Studies Depression scale; DBP, diastolic blood pressure; HRQL, health-related quality of life; PSQI, Pittsburgh Sleep Quality Index; SBP, systolic blood pressure; VR, variance ratio. Mean bias = 15.4%.

Table 21.2: Characteristics of HAART-exposed and HAART-naïve patients in the final propensity score matched sample

Variable	HAART-exposed (N = 229)	HAART-naïve (N = 74)	*P* value	% bias	VR
	Means / Proportions				
Age (years)	37.18	37.68	0.826	-4.4	0.53
Males	29.83 %	28.07 %	0.838	3.9	–
Education > 12 years	21.05 %	21.05 %	1.000	0	–
Working class / unemployed	91.23 %	89.47 %	0.754	4.5	–
Ever smoker	8.77 %	8.77 %	1.000	0	–
Current drinker	35.09 %	28.07 %	0.425	16.2	–
Physical activity (minutes/week)	205.44	205.88	0.991	-0.2	1.33
Body mass index (kg/m^2)	23.41	23.88	0.576	-10.7	0.98
Waist circumference (cm)	84.60	85.11	0.835	-4.3	0.77
Blood glucose (mmol/L)	4.91	4.82	0.701	6.4	0.43
On antihypertensive treatment	1.75 %	1.75 %	1.000	0	–
PSQI	4.46	4.33	0.843	4.0	0.94
CESD	9.42	10.30	0.563	-11.3	0.87
HRQL (physical)	49.03	49.39	0.833	-4.5	1.43
HRQL (mental)	52.68	50.77	0.316	19.7	1.04
Family history of hypertension	12.28 %	12.28 %	1.000	0	–
CD4 cell count (cells/mm^3)	490.07	474.44	0.727	6.8	1.66
HIV infection duration (months)	34.14	35.47	0.776	-5.1	1.36
Baseline SBP (mmHg)	113.96	114.21	0.944	-1.4	1.01
Baseline DBP (mmHg)	76.07	76.14	0.976	-0.6	1.38
Body mass index ## Blood glucose	2069.5	2075.5	0.962	-0.8	0.55

CESD, Centre for Epidemiologic Studies Depression scale; DBP, diastolic blood pressure; HRQL, health-related quality of life; PSQI, Pittsburgh Sleep Quality Index; SBP, systolic blood pressure; VR, variance ratio. Mean bias = 5%

21.3 THE ESTIMATED AVERAGE TREATMENT EFFECTS ON THE TREATED (ATT)

The effect of the treatment (HAART) is the difference between the mean blood pressure for HAART-exposed participants and the mean blood pressure for HAART-naïve participants in the final propensity score matched sample (Rosenbaum & Rubin, 1983). The mean systolic blood pressure levels in the HAART-exposed and HAART-naïve participants in the final propensity score matched sample were 121.25 mmHg and 113.40 mmHg respectively, so that the estimated ATT was 7.85 mmHg, with a standard error (SE) of 3.05 mmHg. The 95% confidence interval for the estimated ATT was calculated using the formula:

95% CI = [1.85 ± 1.96 × SE], where SE = 3.05.

Hence, ATT = [7.85 mmHg (95% CI 3.72 to 15.68)].

Similarly, the mean diastolic blood pressure levels in HAART-exposed and HAART-naïve participants in the final propensity score matched sample were 78.80 mmHg and 71.35 mmHg respectively, so that the estimated ATT was 7.45 mmHg, with SE of 2.2.

Hence, ATT = [7.45 mmHg (95% CI 4.99 to 13.61)].

PART E

DISCUSSIONS OF FINDINGS AND RECOMMENDATIONS

CHAPTER TWENTY-TWO

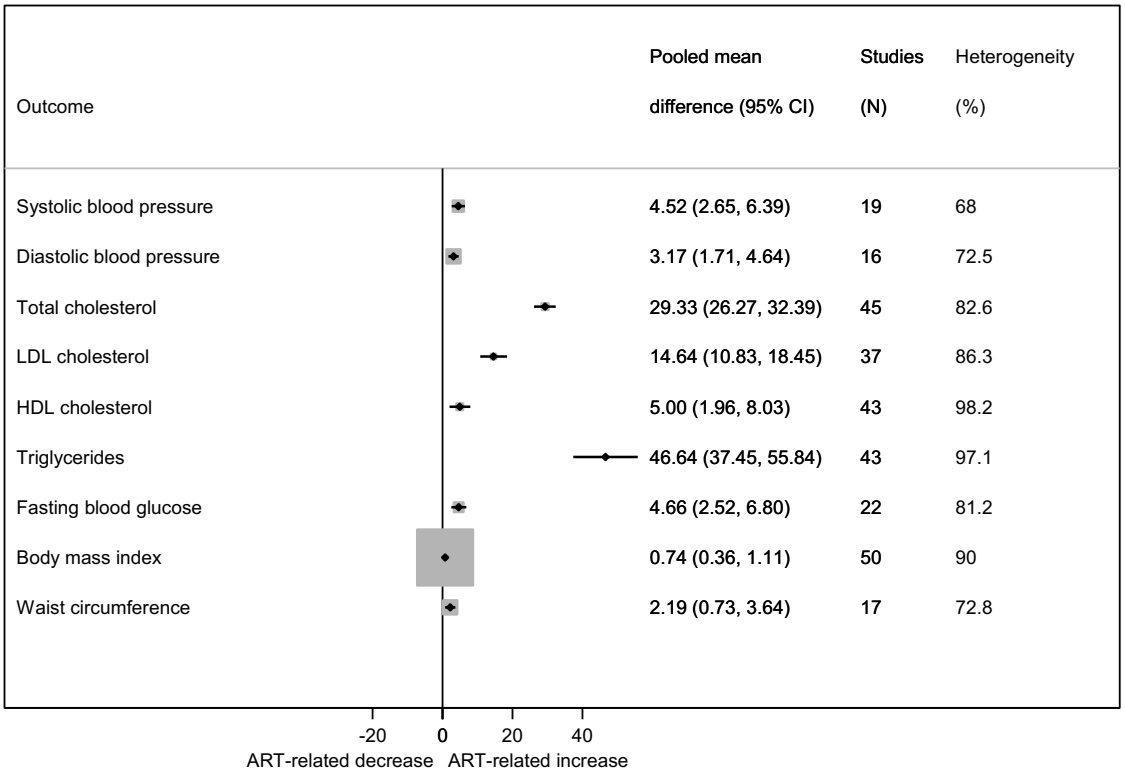
INTERPRETATIONS OF THE MAIN FINDINGS

In this chapter, I interpret the results of the evidence syntheses and primary data analyses.

22.1 INTERPRETING THE MAIN FINDINGS OF GLOBAL EVIDENCE SYNTHESIS

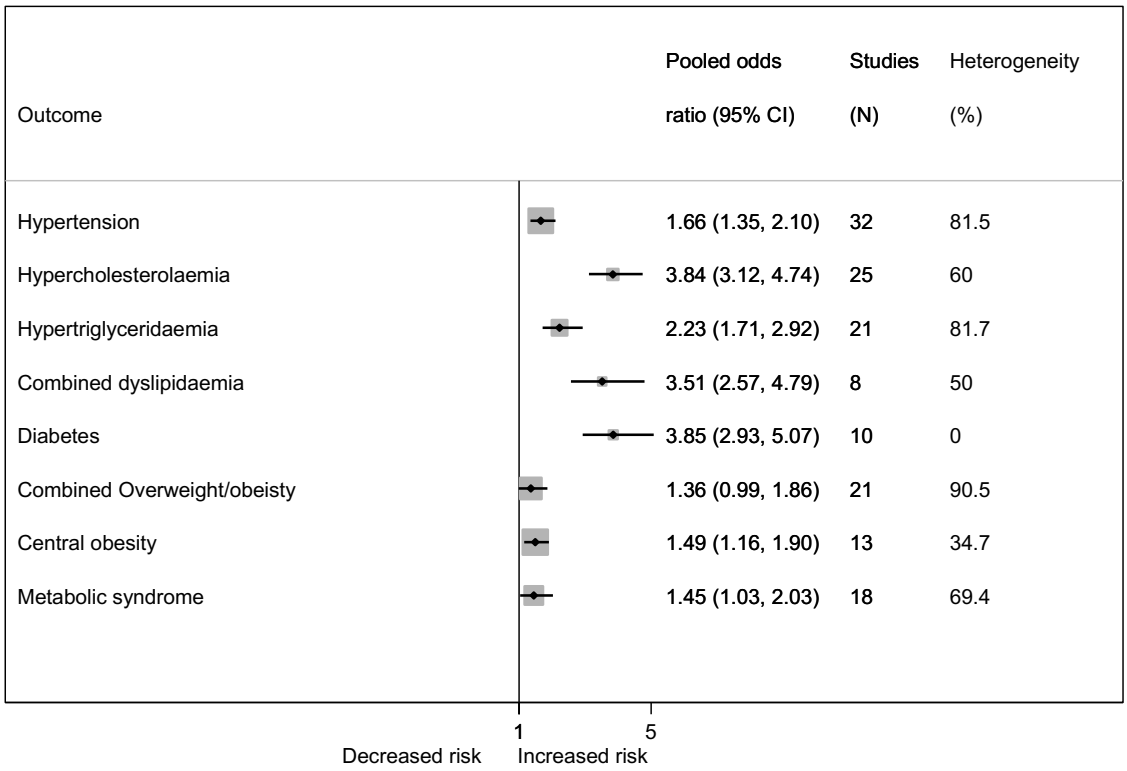
Overall, the findings of the meta-analyses, as summarized in the forest plots below, reveal that antiretroviral therapy is significantly associated with increased blood pressure and other cardiovascular disease risk factors among people living with HIV worldwide.

Figure 22.1: Summary estimates of the pooled associations between antiretroviral therapy and (continuous) cardio-metabolic outcomes



ART, antiretroviral therapy; CI, confidence interval; N, number of studies included in the meta-analyses. Summary plot shows ART is significantly associated with increases in all cardio-metabolic outcome measures.

Figure 22.2: Summary estimates of the pooled associations between antiretroviral therapy and (categorical) cardio-metabolic outcomes



CI, confidence interval; N, number of studies included in the meta-analyses. With the exception of combined overweight/obesity, ART is significantly associated with increased risk of cardio-metabolic disorders.

22.1.1 Antiretroviral-associated increases in blood pressure and hypertension risk

Exposure to antiretroviral therapy was associated with significantly higher blood pressure levels and increased risk of hypertension in HIV-infected patients. With the exception of between-study differences in the proportions of smokers, there was no indication of differential effects — by the different study-level subgroups — in the associations of antiretroviral therapy with increased blood pressure or hypertension risk. For instance, the strength of the association between antiretroviral therapy and increased diastolic blood pressure was significantly greater across studies that reported higher proportions of smokers than non-smokers, compared to studies with fewer smokers than non-smokers, which suggests that smoking could be a moderator of the effect

of antiretroviral therapy on diastolic blood pressure in HIV-infected patients. Schoenbach (2000) and Kamangar (2012) also defined moderation (or effect-modification) in qualitative terms: an association exists between two variables in one subgroup but not in the other, or one subgroup reports an increase in risk but the other subgroup reports a decrease in risk. Drawing on this definition, the subgroup meta-analyses revealed qualitative modifying effects of age, CD4 cell count, duration of HIV infection, and duration of antiretroviral therapy on the impact of antiretroviral therapy on blood pressure. For instance, antiretroviral therapy was significantly associated with increased blood pressure in HIV-infected patients who were above 40 years of age, with CD4 cell counts below 350 cells/mm^3, or had been living with HIV infection for less than five years; whereas no significant associations were observed among their counterparts who were below 40 years of age, with higher CD4 cell counts and longer durations of HIV infection. The significant associations of antiretroviral therapy with increased blood pressure and hypertension risk among studies with mean duration of antiretroviral treatment of 18 months or greater, compared to the lack thereof among studies with mean antiretroviral treatment duration of less than 18 months, suggests that endothelial damage mediated by antiretroviral drugs may be dose-dependent. Although this hypothesis may have alluded to a possible dose-response relationship between antiretroviral exposure and increased blood pressure, one cannot rule out the limited number of small-sized studies with mean antiretroviral treatment duration of less than 18 months, so that this subgroup analysis may have been underpowered to detect a significant association between antiretroviral therapy and blood pressure / hypertension risk.

Meta-regression analysis also provided limited explanation for the observed heterogeneity in the pooled association between antiretroviral therapy and the odds of hypertension: the considerable amount of observed heterogeneity was not explained by any of the study-level characteristics. It is possible that the unexplained heterogeneity may be the results of individual-level differences,

such as: varying individual blood pressure levels prior to the initiation of antiretroviral therapy, differences in antihypertensive treatment accompanied by potential variations in pharmacokinetic interactions with antiretroviral drugs (UCSF Centre for HIV Information, 2014), and varying durations of antihypertensive treatment. However, these data were not available in the included studies, so that individual-level differences could not be investigated in the meta-analyses.

22.1.2 Antiretroviral-associated increases in serum lipoprotein levels and the risks of dyslipidaemias

Antiretroviral therapy was associated with significant increases in serum cholesterol and triglyceride levels among HIV-infected patients. In patients with markedly reduced serum cholesterol levels, antiretroviral-associated increases in serum lipoprotein cholesterol levels may indicate a return to health as opposed to an atherogenic process (Moutzouri, Elisaf & Liberopoulos, 2011). However, the meta-analysis also revealed significant associations between antiretroviral therapy and dyslipidaemias. The observed associations of antiretroviral therapy with increased serum cholesterol and triglyceride levels were somewhat consistent across the different study-level characteristics, albeit with notable differences in the strengths of the associations between certain subgroups given that country income, age, smoking status, CD4 cell count and antiretroviral regimen were determined to be quantitative moderators of the effects of antiretroviral therapy on serum cholesterol levels in HIV-infected patients. For instance, serum cholesterol levels were significantly higher among antiretroviral-exposed patients who were from high-income countries, above 40 years of age, current smokers, with suboptimal CD4 cell counts, and on protease inhibitor-based antiretroviral treatment regimens, compared to antiretroviral-exposed patients who were from low- and middle-income countries, less than 40 years of age, non-smokers, with higher CD4 cell counts, and on reverse transcriptase inhibitor-based antiretroviral treatment regimens respectively. Serum triglyceride levels among antiretroviral-

exposed patients resident in low- and middle-income countries, under 40 years of age, with high CD4 cell counts, on reverse transcriptase inhibitors, and non-smokers were also significantly higher than those of their (antiretroviral) naïve counterparts. Antiretroviral therapy was also the single most consistent determinant of serum triglyceride changes in HIV-infected patients despite variations in the pooled association by geographical region, smoking status, and antiretroviral treatment regimen.

With the exception of the quantitative moderating effects of CD4 cell count, which accounted for 31% of between-study variability in the association of antiretroviral therapy with increased risk of hypertriglyceridaemia, meta-regression analyses provided limited explanation for the observed heterogeneity in the impact of antiretroviral therapy on the dyslipidaemias overall. However, the qualitative modifying effects of antiretroviral treatment duration on these associations are worth emphasizing: it was observed that the risks of hypercholesterolaemia and hypertriglyceridaemia only emerged in HIV-infected patients who had received no less than 18 months of antiretroviral therapy, whereas no significant associations were observed among HIV-infected patients who had been on antiretroviral therapy for less than 18 months. One could argue that this observed interaction may also allude to a possible dose-response relationship between antiretroviral exposure and the risk of dyslipidaemia in HIV-infected patients.

Certain study-level characteristics not included in the meta-regression analyses may have accounted for some of the unexplained heterogeneity in the pooled associations. For instance, individual-level factors, such as individual differences in dietary habits, may have explained partially the observed heterogeneity across the included studies, given that increased dietary fat intake may also be a contributing factor to the high prevalence of cardio-metabolic disorders in people living with HIV (Jaime *et al*., 2006; Joy *et al*., 2007).

Unexpectedly, it was observed that antiretroviral therapy was significantly associated with raised serum levels of HDL cholesterol in HIV-infected patients. HDL cholesterol, also considered 'good cholesterol', helps to lower serum LDL cholesterol levels (Mayo Clinic, 2012). The association of antiretroviral therapy with increased HDL cholesterol levels may have been explained by the potential influence of lipid lowering drugs, such as the statins and fibrates, which may be as effective in improving HDL cholesterol levels as they are in lowering concentrations of the atherogenic LDL cholesterol (Mayo Clinic, 2012). In addition, it is widely acknowledged that lifestyle factors — notably regular physical activity — are the single greatest contributors to increased serum HDL cholesterol concentrations in the general population (Mayo Clinic, 2012). It is possible that physical activity levels in the study population may have influenced the observed antiretroviral-associated increases in mean HDL cholesterol levels. The mechanism to account for this hypothesis relies on the relatively young demographic of the study population, given that most of the participants in the included studies were less than 40 years of age, so that they may have been more likely to be physically active than inactive.

Interestingly, the quantitative modifying effects of the year of publication on the association between antiretroviral therapy and serum cholesterol level suggests evidence of a secular trend. The mechanism accounting for a significantly greater strength of association between antiretroviral therapy and serum cholesterol levels among studies published prior to 2010, compared to studies published from 2010 onwards, are highly speculative at this time, but may rely on a growing awareness and surveillance of the dyslipidaemic effects of antiretroviral therapy in people living with HIV worldwide.

22.1.3 Antiretroviral-associated increases in fasting blood glucose concentration and diabetes mellitus risk

Overall, antiretroviral therapy was significantly associated with increased fasting blood glucose concentration. In addition, HIV-infected patients on antiretroviral therapy had approximately four greater odds of developing diabetes mellitus, compared to patients who were naïve to antiretroviral therapy. There were no statistically significant differences in the pooled associations across different socio-demographic and clinical characteristics, which suggests that antiretroviral treatment may potentially be the single most consistent determinant of blood glucose changes and diabetes risk in people living with HIV. While heterogeneity was statistically significant across the included studies, meta-regression analysis provided very limited explanation regarding the potential sources of heterogeneity. Although, differences between studies in mean CD4 cell counts accounted for the largest amount of variability in the association between antiretroviral therapy and fasting blood glucose levels, only 12% of between-study variability was explained. The observed association of antiretroviral therapy with increasing blood glucose levels in HIV-infected patients on antiretroviral treatment for longer than 18 months but not in patients with shorter treatment durations may suggest a plausible dose-response relationship between antiretroviral exposure and blood glucose levels.

Some of the unexplained heterogeneity may be driven by individual-level differences, such as individual differences in blood glucose concentrations prior to commencing antiretroviral therapy, varying histories of antidiabetic treatment, potential variations in the pharmacodynamic interactions between antiretroviral drugs and oral hypoglycaemic agents (Bakare-Odunola *et al*., 2008), and individual differences in lifestyle behaviours, including alcohol abuse, which may aggravate the hepatotoxic effects of certain antiretroviral drugs, resulting in impaired glucose metabolism (Barve *et al*., 2010).

Furthermore, among the studies that provided data on HIV infection staging, about a quarter of patients presented with AIDS-defining illnesses, which may broadly suggest a potential

modifying effect of the immune reconstitution inflammatory syndrome (IRIS) on antiretroviral-associated increases in blood glucose concentration and diabetes risk. In severely immunocompromised individuals, such as HIV-infected patients presenting with AIDS-defining illnesses, the initiation of antiretroviral treatment overstimulates the immune system, which may be associated with several cardio-metabolic changes, including high blood glucose levels (Mingote *et al*., 2013).

22.1.4 Antiretroviral-associated increases in relative weight and body fat distribution

Overall, the meta-analyses revealed that antiretroviral treatment was associated with significant increases in measures of relative weight (body mass index) and body fat distribution (waist circumference). It is unlikely that these findings reflect a return to health, given that the results also showed antiretroviral therapy to be associated with increased risks of generalized and central obesity. Factors that significantly influenced the associations between antiretroviral therapy and body fat changes include: geographical region of origin of the participants, CD4 cell count, antiretroviral regimen, duration of antiretroviral therapy, and study design. For instance, antiretroviral therapy was significantly associated with increased body mass index and risks of generalized and central obesity among HIV-infected patients from the sub-Saharan African region, but not among HIV-infected patients from other regions, suggesting that genetic trait (proxy by geographical region) might exert a strong modifying effect on the association between antiretroviral therapy and body fat changes. Similarly, CD4 cell counts and antiretroviral regimens were moderators of the effect of antiretroviral therapy on body mass index: HIV-infected patients with CD4 cell counts below 350 cells/mm^3 and patients on non-nucleoside reverse transcriptase inhibitors were substantially at greater risk of antiretroviral-associated relative weight gain, compared to patients with higher CD4 cell counts and patients on protease inhibitor-based antiretroviral regimens respectively. Interestingly, the strength of the association

between antiretroviral therapy and body mass index was significantly greater among HIV-infected patients on antiretroviral therapy for less than 18 months, compared to those on antiretroviral therapy for greater than 18 months; however, this difference only accounted for 9% of between-study variability. Variations by study design in the pooled association between antiretroviral therapy and body mass index may be explained by the substantially higher proportions of participants in cohort studies, compared to cross-sectional studies. Sex also had a moderating effect on the impact of antiretroviral therapy on body fat changes, but only in qualitative terms: the associations of antiretroviral therapy with increased body mass index and risks of generalized and central obesity were significant among women, but not among men. Regional differences and variations in mean CD4 cell counts between studies accounted for most of the between-study variability in the pooled associations between antiretroviral therapy and body fat changes.

22.1.5 Antiretroviral-associated increase in the risk of metabolic syndrome

Antiretroviral therapy was significantly associated with increased risk of metabolic syndrome. HIV-infected patients on antiretroviral therapy had about 1.5 greater odds of having metabolic syndrome, compared to their naïve counterparts. Meta-regression analysis identified age, sex and income setting to be qualitative modifiers of the pooled association: antiretroviral therapy was significantly associated with increased risk of metabolic syndrome among patients above 40 years of age, women and patients from low- and middle-income settings, but not among younger patients, men and patients from high-income settings.

22.2 INTERPRETING THE MAIN FINDINGS OF PRIMARY DATA ANALYSIS

Overall, this study on a sample of HIV-infected adults in a clinic setting in semi-urban Nigeria presents evidence suggesting a high likelihood that a causal link exists between antiretroviral

therapy and increased blood pressure.

22.2.1 The indirect effects of antiretroviral therapy on blood pressure through waist circumference and body mass index.

The findings revealed alternative causal pathways between antiretroviral therapy and increased systolic and diastolic pressure through increases in body mass index and waist circumference. Precisely, the study emphasized the stronger impact of central fat distribution (measured by waist circumference), compared with relative weight (measured by body mass index), in mediating the effects of antiretroviral therapy on systolic and diastolic blood pressure, independent of factors that could attenuate these associations (such as age, sex, lifestyle, CD4 cell count and duration of HIV infection). Besides adjusting for potential confounders, additional measures of inferring a causal link entailed rejecting alternate explanations to the mediational models, such as reverse causality and antiretroviral treatment status as an effect-modifier of the association of body fat measures with blood pressure changes. Although blood glucose levels and sleep quality were not mediators of the associations of antiretroviral therapy with increasing systolic or diastolic blood pressure, it was interesting to observe the significant modifying effects of sleep quality on these associations even after controlling for all pre-selected potential confounders.

22.2.2 The average treatment effect (ATT) of antiretroviral therapy on blood pressure.

Secondly, the estimated average treatment effects on the treated (ATT) revealed that antiretroviral therapy remained significantly associated with increases in systolic and diastolic blood pressure after achieving a balanced distribution of baseline characteristics based on propensity score matching of these characteristics between patients naïve and exposed to antiretroviral treatment.

22.2.3 The impact of antiretroviral treatment status on the clinical prediction of

hypertension in HIV-infected patients.

The results from the model fit statistics were also important because an improvement in the prediction of hypertension following the addition of antiretroviral treatment status to the predictive model was considered an essential foundation for a potential causal impact of antiretroviral therapy on increased blood pressure (Allison, 2014; Cox, 2012). While age, body mass index, family history of hypertension and sleep quality offered the most rigorous method for predicting hypertension in HIV-infected patients in a sub-Saharan African setting, the inclusion of antiretroviral treatment status to the predictive model further improved the clinical prediction of hypertension. To reiterate, improvement in the prediction of hypertension following addition of antiretroviral treatment status reflected the adjusted difference in the risk of hypertension between patients naïve and exposed to antiretroviral therapy. This puts into perspective the progressively higher increase in the predicted probability of hypertension in antiretroviral-exposed patients, compared to antiretroviral-naïve patients, overall and at fixed values of the individual covariates in the predictive model.

CHAPTER TWENTY-THREE

COMPARISON WITH PREVIOUS EVIDENCE

In this chapter, I compare the findings of evidence synthesis and primary data analysis with previous studies.

23.1 COMPARISON OF THE EVIDENCE SYNTHESIS WITH PREVIOUS STUDIES

Although, the observed associations of antiretroviral therapy with increased systolic and diastolic blood pressure were not consistent with the findings of a previous meta-analysis (Dillon *et al*., 2013), it is worth emphasizing that the latter presented evidence only from studies conducted in sub-Saharan African countries, including six relatively small-sized studies, two of which were unpublished. For these reasons, the observed associations of antiretroviral therapy with increasing blood glucose level and body mass index in the present meta-analyses also contradicted the findings of Dillon *et al*. (2013). Hence, one could not rule out the possibility that the studies included in the meta-analyses conducted by Dillon *et al*. (2013) may have been relatively too small to detect a significant impact of antiretroviral therapy on these cardio-metabolic parameters. Nonetheless, the association of antiretroviral therapy with increasing serum cholesterol concentration in the present meta-analysis is consistent with the findings of Dillon *et al*. (2013) in spite of the limitations of the latter. In fact, Dillon *et al*. (2013) reported a significant association between antiretroviral therapy and LDL cholesterol concentrations in people living with HIV in sub-Saharan African countries, which is in line with the antiretroviral-associated increase in LDL cholesterol concentrations observed in the present subgroup meta-analysis of studies conducted in the sub-Saharan African region.

The observed impact of smoking on the association between antiretroviral therapy and diastolic blood pressure is consistent with recent evidence that found heavy cigarette smoking to be associated with increasing diastolic blood pressure and a high prevalence of diastolic hypertension (Dong-Qing *et al*., 2014). The interaction between antiretroviral therapy, smoking and blood pressure may be explained by a potential dual effect of antiretroviral therapy and smoking on blood pressure, which is also coherent with previous studies suggesting endothelial dysfunction as one of the mechanisms by which smoking and antiretroviral therapy increase blood pressure (Ambrose & Barua, 2004; Benowitz, 2003; Celermajer *et al*., 1993; Dau & Holodniy, 2008; Donati, Cauda & Lacoviello, 2010; Messner & Bernhard, 2014; Puranik & Celermajer, 2003).

Previous evidence of the independent associations of ageing and decreasing levels of CD4 cell-mediated immunity with high blood pressure among antiretroviral-exposed HIV-infected patients (Palacios *et al*., 2006) supports the observed moderating effects of age and CD4 cell count on the impact of antiretroviral therapy on blood pressure. In their cohort of 542 HIV-infected patients, Manner *et al*. (2010) reported an inverse association between HIV infection duration and blood pressure, which is consistent with the moderating effects of HIV infection duration on the antiretroviral-associated increase in blood pressure observed in the present meta-analysis.

Seaberg *et al*. (2005) reported a direct relationship between the duration of antiretroviral therapy and blood pressure, which supports the proposed hypothesis of a dose-response relationship between antiretroviral exposure and blood pressure.

A case could also be made for a dual effect of antiretroviral therapy and tobacco smoking on the worsening serum lipid profiles of antiretroviral-exposed HIV-infected patients who smoke. This hypothesis, which potentially explains the stronger associations of antiretroviral therapy with increased serum cholesterol and triglyceride concentrations in studies with more smokers than non-smokers, as opposed to studies with fewer smokers than non-smokers, is compatible with evidence alluding to chronic inflammation and increased oxidative stress as important mechanisms by which smoking and antiretroviral therapy might alter serum lipid levels (Calvo *et al*., 2015; Fleischman *et al*., 2007; Harrison *et al*., 2011; López *et al*., 2004; Stein, 2003).

In a study aimed at examining the impact of ageing on antiretroviral-associated comorbidities among people living with HIV, Orlando *et al*. (2006) found that serum cholesterol, triglyceride and glucose concentrations remained substantially higher in older HIV-infected patients, compared to younger controls, before and after the initiation of antiretroviral therapy — findings consistent with the present meta-analyses. In addition, the direct associations of serum cholesterol and triglyceride concentrations with the duration of antiretroviral therapy (Orlando *et al*., 2006) are somewhat in line with the higher risks of hypercholesterolaemia and hypertriglyceridaemia following longer periods of antiretroviral therapy revealed in the present meta-analyses.

The significantly greater impact of antiretroviral therapy on serum cholesterol levels found among HIV-infected patients with suboptimal CD4 cell counts, compared to those with higher CD4 cell counts in the present meta-analysis are also broadly consistent with previous evidence suggesting a link between lower CD4 cell counts and deranged lipid profiles in HIV-infected patients (El-Sadr *et al*., 2005).

The higher subgroup estimates of the association between antiretroviral therapy and total cholesterol levels among HIV-infected patients from high-income countries, compared to those from low- and middle-income countries, may partially explain the much stronger impact of protease inhibitors than other antiretroviral regimens on serum lipoprotein levels, given that higher proportions of patients from high-income countries rather than from low-resource countries had been treated with protease inhibitor-based antiretroviral regimens. The greater potential for cardio-metabolic toxicity following the use of protease inhibitors over non-protease inhibitor-based antiretroviral regimens corroborates this assertion (Data Collection on Adverse Events of Anti-HIV Drugs [DAD] Study Group, 2007; Mulligan *et al*., 2000; Stein, 2003). However, it is worth noting that Stanley and Grinspoon (2012) found evidence linking obesity to the use of non-nucleoside reverse transcriptase inhibitors over protease inhibitors, which is also reconcilable with the present findings that revealed treatment using non-nucleoside reverse transcriptase inhibitors leading to greater increases in weight and central fat accumulation, compared to protease inhibitors.

The observation of antiretroviral-associated increases in body fat changes among HIV-infected patients in the sub-Saharan African region, but not among patients in other regions, essentially supports the well-established fact that the burden of HIV infection and its consequences are most severe in the sub-Saharan African region.

In line with the stronger impact of antiretroviral therapy on body fat measures in immunocompromised HIV-infected patients than those with normal levels of cell-mediated immunity, Crum-Cianflone *et al*. (2010) — using secondary analysis of data from 1,001 HIV-

infected patients — found a significant association between decreased CD4 cell counts and weight gain.

The greater strength of association between antiretroviral therapy and increasing body mass index in HIV-infected patients on antiretroviral therapy for less than 18months, compared to those who had been on antiretroviral treatment for longer than 18 months, is consistent with the biphasic pattern of antiretroviral-associated changes in body mass index described in the Swiss HIV Cohort Study, where increase in body mass index within the first year of antiretroviral therapy was substantially larger than the increases observed in subsequent years combined (Hasse *et al*., 2014).

The observed associations of antiretroviral therapy with increased risk of metabolic syndrome among HIV-infected patients above 40 years of age and in studies with more women than men, but not among younger patients or studies with fewer women than men, are broadly consistent with older age and female sex as risk factors of cardio-metabolic disorders in HIV-infected persons exposed to antiretroviral therapy (Dagogo-Jack, 2008; Nguyen *et al*., 2016). In addition, the moderating effects of sex on the association between ART and metabolic syndrome is broadly consistent with higher rates of central obesity among women (Garawi *et al*., 2014), especially given that central obesity remains a constant in the definition of metabolic syndrome (International Diabetes Federation, 2006).

23.2 COMPARISON OF THE PRIMARY DATA ANALYSIS WITH PREVIOUS STUDIES

Unfortunately, the dearth of studies reporting the causal average treatment effects of antiretroviral therapy on blood pressure precluded any comparison of the estimated ATT with previous evidence. However, the reported crude effects of antiretroviral therapy on systolic and diastolic blood pressure were consistent with previous evidence from Ghana (MD = 7.90 mmHg [95% CI 2.18 to 13.62] and 4.90 mmHg [95% CI 1.63 to 8.17] respectively) (Ngala & Fianko, 2013), as well as from other developing countries, including Brazil (MD = 8.75 mmHg [95% CI 5.28 to 12.22] and 5.00 mmHg [95% CI 2.35 to 7.65] respectively) (Arruda-Junior *et al*., 2010) and India (MD = 12.72 mmHg [95% CI 9.10 to 16.34] and 7.57 mmHg [95% CI 5.00 to 10.14] respectively) (Mital *et al*., 2013).

The adjusted indirect effects of antiretroviral therapy on systolic and diastolic blood pressure increases through body fat changes were consistent with a large body of scientific evidence on the predominant role of central adiposity, as opposed to relative weight, in the aetiology of cardio-metabolic disorders (Van Pelt *et al*., 2001; Janssen, Katzmarzyk & Ross, 2004; Kamath, Shivaprakash & Adhikari, 2011; Stranges *et al*., 2004; Stranges *et al*., 2005; Nduka *et al*., 2015b; Yusuf *et al*., 2004). Similarly, the pathophysiological mechanisms to account for the differential mediating effects of body mass index and waist circumference reside mainly in the relatively limited measure of body mass index, compared with waist circumference, in detecting important cardio-metabolic changes driven by central fat accumulation (Janssen, Katzmarzyk & Ross, 2004; Kamath, Shivaprakash & Adhikari, 2011; Yusuf *et al*., 2004).

In ruling out an alternate explanation of the mediational model, the stratified analyses by HAART status may not have been adequately powered to detect effect modification of the associations between body fat measures and blood pressure, given the relatively small sample size of HAART-naïve patients (Kamangar, 2012). However, the absence of effect-modification may also be reconcilable with the chronic inflammatory and platelet activating effects of HIV infection — known mechanisms that underlie blood pressure changes in antiretroviral-naïve HIV-infected patients (Blann *et al*., 1997).

With regards to the model fit statistics, the selection of variables included in the predictive model for hypertension was guided solely by the degree of fit. However, one cannot dismiss the biological connection that exists among these variables and hypertension. For instance, the predictive model was coherent with the interplay between poor sleep quality, ageing, overweight/obesity and hypertension. Essentially, poor sleep quality interferes with metabolism and hormonogenesis, which underlie the aetiology of cardio-metabolic disorders (Bruno *et al*., 2013; Shlisky *et al*., 2012; Stranges *et al*., 2008). In addition, the impact of HAART status in improving the clinical prediction of hypertension among HIV-infected patients is corroborated by evidence on the biological plausibility of this link, such as the endothelial damaging effects of antiretroviral drugs (Dau & Holodniy, 2008; Stein, 2003).

CHAPTER TWENTY-FOUR

LIMITATIONS AND STRENGTHS

In this chapter, I discuss the limitations and strengths of evidence synthesis and primary data analysis. I also highlight the limitations of the mini-review.

24.1 LIMITATIONS AND STRENGTHS OF THE GLOBAL EVIDENCE SYNTHESIS

The observational nature of the data precluded any causal inferences; however, treating HIV-infected patients as controls being administered placebos in a randomized controlled trial would have posed considerable ethical challenges.

With more than 60% of the included studies assessed to have a high (or unclear) risk of selection bias, the methodological quality across the included studies may have been moderate at best. The high risk of selection bias observed across the included studies was not unexpected, given the observational nature of the evidence synthesis. Although meta-regression analyses revealed that selection bias had no significant impact on the pooled effects of antiretroviral therapy on cardio-metabolic parameters, it is worth emphasizing that meta-analyses are only as good as the included studies, and are prone to the same biases as the including studies. The present meta-analyses included mostly cross-sectional studies, which were inherently prone to selection bias. The absence of randomisation in the included studies meant that HAART-exposed and HAART-naïve patients were systematically different. For instance, HAART-exposed and HAART-naïve patients presenting to HIV clinics may have differed in their durations of HIV infection, where HAART-exposed patients were more likely to have been exposed to the effects of HIV infection for a much longer duration than their naïve counterparts. HAART-exposed patients had significantly lower CD4 cell counts, compared to their naïve counterparts, which may not be unrelated to the observed disparity in HIV infection duration between both groups,

given the widely acknowledged inverse association between viral load and CD4 cell count. HAART-exposed patients were significantly older than HAART-naïve patients. Men were more likely to be HAART-exposed, whereas women were more likely to be HAART-naïve. Smoking status also differed by HAART status. In addition to the observed differences in the data, HAART-exposed and HAART-naïve patients characteristically have different times to follow-up, where the intervals between follow-up visits are longer for HAART-exposed patients than they are for HAART-naïve patients. HAART status may have also been influenced by proximity to the clinic, suggesting that HAART-exposed and HAART-naïve patients may have differed in their places of residence, and potentially accounting for the higher proportion of HAART-exposed patients compared to HAART-naïve patients in the study: a likely occurrence in low- and middle-income countries.

The systematic differences between HAART-exposed and HAART-naïve patients in the included studies also meant that there was a high potential that the pooled estimates were confounded. For instant, the modifying effect of smoking on the pooled association between HAART exposure and increased blood pressure may have been the result of significant differences in the proportions of smokers between HAART-exposed and HAART-naïve patients, and may also suggest that smoking was a potential confounder of the pooled effects of HAART on blood pressure. The differences in the proportions of smokers between HAART-exposed and HAART-naïve subjects also accounted for the largest amount of heterogeneity in the study-by-study estimates of the effects of HAART on blood pressure, again suggesting that the pooled mean differences in blood pressure between HAART-exposed and HAART-naïve patients may be confounded by differences in proportions of smokers between both patient groups. Although smoking was incorporated in the meta-regression models to account for its impact on the pooled effects of HAART on blood pressure and other cardio-metabolic parameters, it is also important to emphasize that the other main problems associated with

confounding. For instance, there was a high potential for residual confounding as not all important confounders were accounted for in the included studies, probably because the investigators were unaware of them. For example, other lifestyle factors such as alcohol abuse, physical inactivity and poor dietary habits were not reported in most of the included studies. In addition, even when these potential confounders were identified in some of the included studies, they were not measured objectively (Shrier *et al*., 2007). For instance, assays for biomarkers such as cotinine and Gamma-glutamyltransferase are more accurate and more sensitive than self-reported measures for quantifying smoking and alcohol consumption levels respectively. Similarly, motion sensors (such as pedometers) may provide more objective estimates of physical activity levels, compared to self-reports. However, these investigations were not performed, potentially suggesting that the pooled estimates may have been confounded by these lifestyle factors.

Furthermore, at the time of enrolment into care and treatment, HIV-infected patients who are eligible to receive HAART may have been more susceptible to blood pressure changes if compared to HIV-infected patients who were not eligible to receive HAART because of the presence of risk factors at baseline (prior to HAART exposure). For instance, patients eligible to commence antiretroviral therapy may have had longer duration of infection and lower CD4 cell counts, and may have been more likely to smoke or consume heavy amounts of alcohol, compared to patients not eligible for antiretroviral treatment. HIV-infected patients eligible for antiretroviral therapy at the time of HIV infection diagnosis may have also been older than those not eligible for antiretroviral therapy, given the effect of ageing on HIV disease progression (Edwards *et al*., 2015).This source of confounding which is linked to HAART status has been termed susceptibility bias (Delgado-Rodríguez & Llorca, 2003), as the effect estimates may have been biased from the onset in favour of the HAART-exposed group.

Less than 20% of the total study population comprised people living in low- and middle-income countries — where the burden of HIV infection is most severe, and increases in antiretroviral treatment coverage rates are the steepest. This disparity may reduce the generalizability of the findings across different geographic and socio-economic settings.

The unexplained heterogeneity in the pooled estimates may be attributed to study-level factors not considered in the meta-analyses, but only because these factors were seldom assessed in the individual primary studies. For instance, it was not known whether differences in antihypertensive, lipid-lowering, or hypoglycaemic treatments accounted for some of the between-study variability in the effect estimates.

Meta-regression analyses to determine potential effect-modifiers of the associations of antiretroviral therapy with increased risks of combined dyslipidaemia and diabetes mellitus were not performed, given that the studies reporting these outcomes were insufficient for the meta-regression coefficients to be estimated effectively (Thompson & Higgins, 2002).

The dearth of studies reporting on lifestyle factors meant that the effects of poor dietary habits, alcohol abuse and physical inactivity on the pooled associations between antiretroviral therapy and cardio-metabolic changes could not be ascertained. For instance, the author had planned to examine the potential impact of alcohol abuse on the effect of antiretroviral therapy on blood glucose concentration, especially given the higher prevalence of alcohol abuse among people living with HIV, compared to the general population, as well as the potential for mediating a range of hepatitides that tend to precede the impairment of glucose metabolism (Barve *et al*., 2010). Furthermore, the interaction between heavy alcohol consumption and antiretroviral

exposure has been shown to increase hepatitis C virus RNA levels (Cooper & Cameron, 2005). However, data on co-infection between HIV and other blood-borne viruses were not reported in the vast majority of the included studies.

More than a third of patients in studies providing data on HIV infection staging presented with one or more AIDS-defining illnesses. However, studies reporting AIDS-defining illnesses for each meta-analysis were also insufficient for meta-regression analyses to converge and be estimated effectively (Thompson & Higgins, 2002), potentially missing out on any information regarding the impact of HIV clinical staging on the pooled associations between antiretroviral therapy and cardio-metabolic conditions.

The impact of antiretroviral adherence on the pooled effects of antiretroviral therapy on the cardio-metabolic parameters was not assessed because none of the included studies reported antiretroviral treatment adherence levels for the participants. The importance of such an assessment is exemplified in the Strategies for Management of Antiretroviral Therapy (SMART) trial, which revealed a substantially higher risk of cardiovascular disease among HIV-infected patients in whom antiretroviral treatment was interrupted, compared to HIV-infected patients on continuous antiretroviral treatment (SMART Study Group, 2006).

The effect of antiretroviral therapy on glycated haemoglobin concentrations (HbA1c) could not be explored in the present meta-analysis. As cited in Dillon *et al*. (2013), no more than two unpublished studies have compared HbA1c levels between HIV-infected patients naïve and exposed to antiretroviral therapy. The dearth of primary studies on this biomarker in HIV-infected subjects is an important limitation of the present systematic review and meta-analysis, especially

with HbA1c now considered to be a better predictor of long-term diabetes risk than fasting plasma glucose in the general population (Selvin *et al*., 2010).

The lipoprotein ratios, such as total cholesterol/HDL cholesterol ratio and triglycerides/HDL cholesterol ratio, are considered to be better predictors of cardiovascular risk, compared to conventional lipid profile (Millan *et al*., 2009; Tamang *et al*., 2014). However, the dearth of studies examining the impact of antiretroviral therapy on lipoprotein ratios precluded this investigation.

Lastly, in assessing the effects of antiretroviral therapy on body fat changes, more sensitive measures of body fat, such as fat mass, were not used because of the dearth of studies reporting these body fat measures.

In spite of the above limitations, the strengths of this systematic review and meta-analyses are important with regard to the novelty and methodological rigour used to arrive at the findings.

First, the author presents the most comprehensive evidence and first pooled analyses examining the association of antiretroviral therapy with a range of cardio-metabolic parameters in people living with HIV, thus filling an important gap in the literature.

Secondly, subgroup and meta-regression analyses were performed to identify interrelated and often interacting factors that influence qualitative and quantitative cardio-metabolic changes in people living with HIV on antiretroviral therapy; the findings from these analyses also represent evidence to advance the field.

The author conducted comprehensive searches of electronic databases to ensure that all relevant publications were identified. In addition, any potential bias in the conduct of this systematic review was minimized when the co-authors (supervisors) independently scanned through the search output and vetted the extracted data.

Tests for publication bias revealed no evidence of small-study effects that could potentially invalidate the findings, and no indication of potentially missing studies that could have altered the interpretations of the pooled associations.

Leave-one-out sensitivity analyses also revealed no indication of undue influence by specific studies as to changing the interpretations of the pooled associations.

24.2 IMITATIONS AND STRENGTHS OF THE MINI-REVIEW

The limitations of the mini-review essentially entail the drawbacks of using Hill's criteria as an instrument for examining causality. For instance, Hill (1965) and Rothman (2002) argue that the only absolute essential criterion for establishing a causal inference is the temporal relationship between the exposure and the outcome. Rothman (2002) also implied that the other criteria for causation were somewhat non-essential. Although these criteria were never intended to be absolute, they do provide an essential framework for establishing cause and effect (Lucas & McMichael, 2005).

Secondly, it is important to emphasize that Hill's theories about causality were informed at a time which coincided with the early discoveries within the field of chronic non-communicable disease

epidemiology. Expectedly, the fundamental objective at the time was the study of simple, direct causation between an exposure and an outcome (Mirtz *et al*., 2009), and not so much the complex interplay of risk factors that characterise disease processes today.

Furthermore, Hill's criteria of causation do not incorporate complex statistical concepts that actually enhance the definition of causality (Austin, 2011a; Henneken & Buring, 1987; Kenny, 2015).

24.3 LIMITATIONS AND STRENGTHS OF THE PRIMARY DATA ANALYSIS

The findings must be interpreted with caution, given the observational nature of the study and the potential for residual confounding and bias, which have been discussed previously (section 24.2): the issues relating to bias and confounding in the evidence synthesis are also common drawbacks of primary epidemiological studies in low-resource settings.

Importantly, caution must be applied in interpreting causal effects. Hume (2000) argued that causality was a term open to interpretation in the sense that it was an inference based on a logical assumption, and not an entity based on empirical evidence. In other words, regardless of the design of a study, one could never absolutely ascertain that an exposure (such as antiretroviral therapy) caused a disease (such as high blood pressure). Nevertheless, Buck (1975) suggested that applying caution in the interpretation of a causal inference should entail the rejection or modification of a causal hypothesis, and not the proof of causation.

The study was set in semi-urban Nigeria, which may have limited generalizability of the findings to HIV-infected populations from rural settings and outside the sub-Saharan African region. For example, regional variations exist in the age groups of HIV-infected populations, as people living with HIV in sub-Saharan African countries tend to be generally younger than those resident in

less deprived settings (UNAIDS, 2013). In addition, while the reverse transcriptase inhibitors are the mainstay of antiretroviral therapy in sub-Saharan African countries (Bloomfield & Velazquez, 2013; Federal Ministry of Health Nigeria, 2010; Ministry of Medical Services Kenya, 2011), protease inhibitors are the drugs of choice for initiating antiretroviral treatment among people living with HIV across European countries and the Americas (Hughes *et al*., 2011); hence, different classes of antiretroviral drugs may confer differential effect measures on body fat and blood pressure changes in people living with HIV.

Obtaining dietary data from the study participants was fraught with considerably high levels of recall bias, so that the potential influence of diet on the effects of HAART on blood pressure was not assessed. However, it is important to emphasize that lifestyle factors such as poor dietary habits may be of limited value in predicting blood pressure changes in people living with HIV on antiretroviral therapy (Troll, 2011).

Household income has been identified as a better predictor of health outcomes when compared to other known socioeconomic indicators such as education and occupational grade (Daly *et al*., 2002). However, the use of household income as a socioeconomic indicator of health in the study was limited by differences in access to the family income among household members, as is often the case in resource-limited settings (Daly *et al*., 2002). For instance, women were more likely than men to have limited access to household income. In addition, household income would not adequately account for the social positions of retirees who do not earn monthly incomes, but may possess a substantial amount of financial resources (Daly *et al*., 2002). Expectedly, a considerable proportion of the participants were also wary about disclosing their income.

The indirect effects of antiretroviral therapy on blood pressure changes through more sensitive

measures of adiposity (such as fat mass) could not be ascertained: the assessment of body fat mass would have entailed the use of sophisticated and costly investigative techniques, including dual energy x-ray absorptiometry (DEXA) and bioelectrical impedance analysis (Donini *et al*., 2013), which are often not feasible in sub-Saharan African settings, given resource constraints. Similarly, 24-hour ambulatory blood pressure monitoring, which represents the gold standard for the clinical diagnosis of hypertension (National Institute for Health and Care Excellence, 2011), was not feasible in our study setting, given resource constraints.

Unfortunately, the impact of serum lipoprotein levels on the effects of antiretroviral therapy on blood pressure changes could also not be ascertained given resource constraints. Such an analysis could have provided evidence of alternative mediating mechanisms of antiretroviral therapy on increased systolic and diastolic blood pressure through changes in serum lipoprotein levels, as supported by previous landmark studies (Bonna & Thelle, 1991; Hjermann *et al*., 1978; Dillon *et al*., 2013; Nduka *et al*., 2015b). However, as is the case in many sub-Saharan African countries, lipid profile assessments are rather expensive and are not subsidized by the governments of these countries or donor organisations (Bloomfield & Velazquez, 2013).

The mediating effects of body fat measures on the association between antiretroviral therapy and increased blood pressure may suggest a return to health, as opposed to a pathological process. It could be speculated that this phenomenon would relatively be more likely to occur in HIV-infected patients who were in advanced clinical or immunological stages of HIV infection. For instance, Tate *et al*. (2012) reported significant increases in body mass indices in HIV-infected patients with CD4 cell counts below 50 cells/mm^3 following the initiation of antiretroviral therapy. CD4 cell counts below 50 cells/mm^3 have also been associated with sustained increases in blood pressure following the commencement of antiretroviral treatment (Manner *et al*., 2013).

Such patients, who are likely to be antiretroviral-naïve or resistant to antiretroviral treatment, tend to be underweight and hypotensive, so that the initiation of antiretroviral treatment is likely to improve body weight and blood pressure levels, albeit transiently (Chow *et al*., 2003; Kelly *et al*., 2002). Although antiretroviral-associated increases in blood pressure and body fat measures tend not to be sustained in IRIS, it is still arguable that such changes in cardio-metabolic parameters may be symptomatic of the exaggerated inflammatory response that follows the commencement of antiretroviral therapy in HIV-infected patients with advanced disease (Bosamiya, 2011). However, none of the participants in our study presented with an AIDS-defining illness or was determined to be resistant to antiretroviral treatment, as these two conditions were part of the exclusion criteria for the present study.

Although a positive family history is among the well-established clinical predictors of hypertension, the potential for recall bias could not be dismissed, given that HIV-infected patients in the present study who were assessed as hypertensive were more than six times likely to report a history of hypertension in a first-degree relative. Similarly, smoking and drinking status may have been influenced by high rates of recall bias. Given the self-reported nature of these data, the higher propensity for alcohol and tobacco consumption among people living with HIV compared to the general population (Bonacini, 2011; Savès *et al*., 2003), and the demonization of chronic smoking and drinking in certain sub-Saharan African settings, it is plausible that the proportions of ever smokers and current alcohol users in our study may have been under-estimated, suggesting why these traditional risk factors of hypertension did not meet the criteria for inclusion in the predictive model.

It is also possible that the low amounts of explained variability corresponding to depression and HRQL scores may have reduced the statistical power of these variables (UCLA Institute for Digital Research and Education, 2015a), thus providing some explanation as to why these known

predictors of hypertension (Stranges & Donahue, 2015; Thiébaut *et al*., 2005) were not eligible for inclusion in the predictive model for hypertension.

Despite these limitations, the study presents important strengths. For instance, this study represents the first of its kind that examined the possibility of a causal association between antiretroviral therapy and increased blood pressure. The indirect effects of antiretroviral therapy on increased blood pressure through body fat changes present novel evidence to advance the field.

In demonstrating that the effects of antiretroviral therapy on systolic and diastolic blood pressure were not attributed to any residual differences in the study covariates between patients naïve and exposed to HAART, the estimated ATT from propensity score matching potentially reflects a higher level of evidence and a substantially lower potential for residual confounding, compared to previous observational studies (Austin, 2011a; Seeger, Kurth & Walker, 2007). In fact, the odds of residual confounding or selection bias are hardly any different between analyses of a randomised controlled sample and a propensity score-matched sample (Austin, 2011a).

The sample size of the propensity score-matched sample was powered to detect a significant ATT while accounting for 25% drop out of patients who were not matched on the propensity score. In addition, the methods employed in ensuring that the characteristics of the propensity score-matched sample were adequately balanced between antiretroviral-exposed and naive patients were rigorous and entailed a three-step process based on the differences in the means and proportions of the baseline characteristics, the standardised bias after matching the baseline characteristics on the propensity scores, and the ratio of the variances in both exposure groups (Austin, 2011b).

The *causal inference approach* to mediation was employed to rule out potential influences from omitted variables (or confounders), reverse causality, or alternate explanations to the

hypothesized causal pathway. Of note, estimates of the indirect effects were less likely to be exaggerated, with the inclusion of important covariates as potential confounders in the mediation models.

The exclusion of patients in advanced stages of HIV infection from the study did not allow for an alternate interpretation of the mediation analyses, so that the indirect effects of antiretroviral therapy on systolic and diastolic blood pressure through changes in body mass index and waist circumference were more likely to be pathological than indicative of a physiological return to health.

Bootstrapping the indirect effect estimates with 95% confidence intervals was the preferred alternative to the Sobel-Goodman mediation test in ascertaining the statistical significance of the indirect effects. Compared to the latter, bootstrapping is more robust to sample size, so that the present study was unlikely to produce inaccurate estimates of the indirect effects (Preacher & Hayes, 2008; MacKinnon, Lockwood & Williams, 2004). The 95% confidence intervals of the coefficients of the indirect effects were bias-corrected, potentially controlling for any potential skewness in the data (Cohen, 1988; Fritz & MacKinnon, 2007).

Lastly, several important clinical correlates of blood pressure, which have not been examined previously in connection with the impact of antiretroviral therapy on blood pressure, especially in sub-Saharan African settings, notably were ascertained in the analyses. For instance, the potential influence of certain indicators of psychological and mental wellbeing (such as sleep quality, presence of depressive symptoms and HRQL) on the effects of antiretroviral therapy on blood pressure changes were examined, taking into account the holistic nature of an individual's health and wellbeing (World Health Organisation, 1946).

CHAPTER TWENTY-FIVE

IMPLICATIONS FOR PRACTICE AND FUTURE RESEARCH

In this chapter, I discuss the implications of the evidence synthesis and primary data analysis for public health practice/policy and future research.

25.1 IMPLICATIONS OF THE GLOBAL EVIDENCE SYNTHESIS FOR PRACTICE AND POLICY

The findings of the review may have important clinical and public health implications for people living with HIV worldwide. For instance, the results suggest that even small shifts population blood pressure may have a significant impact on the incidence of hypertension and its complications among the 37 million people living with HIV worldwide (Stamler, 1991; Stamler, Stamler & Neaton, 1993). To this end, people living with HIV may benefit from regular hypertension screening and other cardiovascular risk assessments following the initiation of antiretroviral therapy. However, it is worth noting that evidence from experimental studies are required in order to implement such policy.

Antiretroviral treatment coverage across the sub-Saharan African region has been estimated to be about 39% (UNAIDS, 2014a). In addition, in many sub-Saharan African countries, more than half the people estimated be living with HIV are not aware of their HIV status. Hence, there is great emphasis on tackling these issues through improving HIV testing uptake and antiretroviral treatment coverage rates. However, this comes at the expense of tackling the burden of non-AIDS-defining illnesses, such as the cardio-metabolic sequelae of antiretroviral use, among people living with HIV in sub-Saharan African settings. Cardiovascular diseases continue to be neglected at the population level among people living with HIV in many sub-Saharan African

countries (Bloomfield & Velazquez, 2013), potentially worsening the already-existing burden of non-communicable diseases within the general population in these countries.

Preventive measures ought to be considered in tackling the potential publication health implications of expanding antiretroviral coverage rates, especially in sub-Saharan African countries, where two-thirds of all people living with HIV resides. At the very least, while we await evidence from randomised controlled trials supporting the clinical and cost-effectiveness of hypertension screening and other cardiovascular risk assessments in this high-risk group, hypertension and other cardio-metabolic disorders may be acknowledged as potential public health problems in people living with HIV.

The findings also have implications for the treatment of hypertension and other cardiovascular conditions among people living with HIV. For the majority of people living with HIV worldwide, the treatment of cardiovascular disorders is often administered in isolation from the potential complications of antiretroviral treatment (Bloomfield & Velazquez, 2013). Such treatment effects tend to be transient because they are not being informed by an understanding of the epidemiological mechanisms between antiretroviral therapy and cardiovascular disease risk factors, and because they ignore the potential drug-drug interactions between antiretroviral and anti-hypertensive drugs.

25.2 IMPLICATIONS OF THE GLOBAL EVIDENCE SYNTHESIS FOR FUTURE RESEARCH

The meta-analyses highlight the need for additional evidence to investigate the possibility of a causal association between antiretroviral therapy and cardio-metabolic changes. Given the inevitable ethical ramifications of addressing this question using a randomised controlled trial,

estimating the causal effects of antiretroviral therapy on cardio-metabolic outcomes using observational data may present the only feasible alternative. Statistical methods such as propensity score analyses have become quite popular for estimating causal average treatment effects using observational data (Austin, 2011a), and could be useful in this context. Observing study participants for incident HIV infection, antiretroviral therapy and cardio-metabolic changes may also provide a suitable — albeit challenging — alternative for estimating a potential causal association.

Although, the identification of effect-modifiers in the meta-analyses elucidates the complex epidemiological interactions between antiretroviral therapy and cardio-metabolic changes, effect measure modification models are less appropriate and less powerful than mediation models for testing causal inferences (Kenny, 2015). Therefore, as part of assessing the likelihood that a causal link exists between antiretroviral therapy and cardio-metabolic changes, it would be useful to examine alternative mechanisms that mediate this pathway.

25.2 IMPLICATIONS OF THE PRIMARY DATA ANALYSIS FOR PRACTICE AND POLICY

The findings of this study come at a time of renewed commitment by the United Nations to scale up universal antiretroviral treatment coverage rates (UNAIDS, 2014a). While further reductions in the prevalence of AIDS-defining illnesses and AIDS-specific mortality rates are the expected outcomes of this intervention, little is known about the potential public health implications of a continued global expansion of antiretroviral coverage rates, especially in sub-Saharan African populations. Discussions about the impact of antiretroviral therapy on cardio-metabolic disorders tend to ignore this region of the world where HIV infection is most prevalent. Cardiovascular disease risk factors are becoming exponentially more rampant. Increases in antiretroviral

treatment coverage rates are the steepest of all geographical regions. Concerns that the potential burden of antiretroviral-associated cardio-metabolic disorders may exacerbate the already-growing epidemic of cardiovascular disease are valid (Bloomfield & Velazquez, 2013; Dillon *et al*., 2013; Jamison *et al*., 2006; Nduka *et al*., 2016; Nduka *et al*., 2015c; Sarki *et al*., 2015). However, it is important to reiterate that the withdrawal of antiretroviral treatment is inevitably fatal for HIV-infected patients who are eligible to commence antiretroviral therapy, so that such a measure cannot be an option for prevention against antiretroviral-associated cardiovascular disease risk factors.

The findings potentially support recognition of antiretroviral-associated hypertension as a separate entity from hypertension secondary to other risk factors. The fundamental differences in cardiovascular risk factor profile between people living with HIV and the general population, evidence affirming the limitations of traditional risk factors in predicting antiretroviral-associated cardio-metabolic disorders (Troll, 2011), and the relative inefficacy of standard cardiovascular risk management strategies in high-risk subgroups such as people living with HIV on antiretroviral therapy (Masia *et al*., 2009) are other scientific arguments supporting this recommendation.

The results of the study are important particularly for the care and treatment of people living with HIV in Nigeria and many other countries in the sub-Saharan African region where HIV care and treatment guidelines do not entail comprehensive baseline and routine cardiovascular risk assessments, or surveillance systems for monitoring cardiovascular disease risk factors in this high-risk population (Bloomfield & Velazquez, 2013; Federal Ministry of Health Nigeria, 2012).

With the persistent rise in living costs in Nigeria — and even more so in the current economic and political climate — it becomes necessary for HIV-infected persons needing services for

cardio-metabolic conditions to have free access to these services, just as they have to antiretroviral treatment (Bloomfield & Velazquez, 2013; Federal Ministry of Health Nigeria, 2012).

From a clinical perspective, the findings suggest that the use of waist circumference as a complementary body fat measure to body mass index may improve the clinical prediction of hypertension among HIV-infected patients on antiretroviral therapy. Additionally, the prediction and early detection of high blood pressure in HIV-infected patients using measures of central fat distribution and relative weight may potentially strengthen the surveillance of hypertension and its complications in clinic settings. These are important considerations given that a substantial number of hypertension cases are initially asymptomatic and are marked by considerable rates of underdiagnosis in people living with HIV in sub-Saharan African settings (Bloomfield & Velazquez, 2013).

The findings also imply that interventions aimed at weight management may be effective for blood pressure control among HIV-infected patients. While lifestyle factors may be limited in predicting hypertension and other cardio-metabolic disorders in people living with HIV on HAART (Troll, 2011); therapeutic lifestyle interventions have been found to be of modest benefit in reducing blood pressure in this high-risk subgroup (Balasubramanyam *et al*., 2011).

The mainstay of managing high blood pressure associated with antiretroviral therapy may warrant switching antiretroviral regimens. Antiretroviral switch therapy is commonly used on an individualized basis in hospital settings for managing patients with HAART-associated cardiovascular conditions such as high blood pressure (Calza *et al*., 2005; Rasmussen *et al*.,

2011). However, such treatment options are not readily available in low-resource settings such as Nigeria, resulting in an overdependence on antihypertensive medications, which potentially interact with antiretroviral drugs.

The findings also support the addition of sleep to the cluster of behavioural factors associated with cardiovascular risk among people living with HIV in sub-Saharan African settings.

Lastly, HIV-infected patients who are eligible to commence antiretroviral therapy should be candidates for routine comprehensive cardiovascular risk assessments. The Data collection on Adverse effects of anti-HIV Drugs (DAD) risk calculator has been reported to be a more suitable tool for assessing cardiovascular risk in HIV-infected patients, compared to the Framingham risk assessment tool which tends to underestimate the risk of cardiovascular disease in people living with HIV (Law *et al*., 2006). However, its (DAD risk calculator) potential validity in sub-Saharan African settings may be limited, given that the algorithms were developed for European populations (Friis-Møller *et al*., 2010). The development of algorithms for a cardiovascular risk assessment tool that is feasible within sub-Saharan African contexts could be an important step forward to tackle the burden of cardiovascular disease in people living with HIV in such settings.

25.3 IMPLICATIONS OF THE PRIMARY DATA ANALYSIS FOR FUTURE RESEARCH

Given that this study represents the first of its kind, further studies with larger samples are needed to determine if the present findings would be replicated across different settings.

Whether or not the results are consistent in prospective cohort studies should also be investigated by future studies.

Future studies also need to identify what phenotypes on the HIV clinical spectrum are most susceptible to the effects of antiretroviral therapy on blood pressure and other cardio-metabolic parameters. The efficacies of targeted interventions on these phenotypes should be investigated in clinical trials.

The indirect causal effects of antiretroviral therapy on increasing blood pressure through changes in serum lipoprotein levels and more sensitive measures of body fat, such as fat mass, should also be explored by future studies.

To reiterate, the results support the validation of a cardiovascular risk assessment tool in people living with HIV in sub-Saharan African countries.

While the results do not support the integration of depression screening with cardiovascular risk evaluation of HIV-infected patients in Nigeria; detection of depressive symptoms occurred in one out of every five — previously undiagnosed — HIV-infected patients in this study, which potentially reveals that the burden of psychiatric morbidities and poor mental wellbeing among people living with HIV in sub-Saharan African settings has been a neglected issue in epidemiological research.

CHAPTER TWENTY-SIX

CONCLUSION

In this chapter, I summarise the findings of the dissertation, while reinforcing the main ideas.

26.1 CONCLUSION

The meta-analyses, comprising data on more than 80,000 HIV-infected subjects, suggest that antiretroviral therapy may potentially be the single most consistent correlate of cardio-metabolic changes in people living with HIV worldwide. Older people, current smokers, patients with impaired cell-mediated immunity, and patients who have been exposed to antiretroviral therapy for at least 18 months may be more susceptible to the effects of antiretroviral on blood pressure and other cardiovascular risk parameters, when compared to other HIV-infected population subgroups.

The Hill's criteria are still the most commonly employed epidemiological tool for determining whether a causal link potentially exists between an exposure (or a treatment) and an outcome. In spite of the limitations to using Hill's criteria, the evidence in the literature mostly allude to a plausible causal link between antiretroviral therapy and increased blood pressure.

The primary data analysis revealed a high probability that the association between antiretroviral therapy and increased blood pressure may be causal in nature, being partially mediated by central fat accumulation and relative weight gain. The estimated average treatment effects on the treated of a propensity score-matched sample of the study population also provided evidence suggestive of a plausible causal link between antiretroviral therapy and increased blood pressure. These

analyses are coherent with a growing interest in using observational data to test causal hypotheses, especially in cases where controlled experimentation is not feasible (such as the present study).

Overall, the findings suggest that people living with HIV in sub-Saharan African countries may benefit from regular hypertension screening and other cardiovascular risk assessments, especially after the commencement of antiretroviral therapy. Larger prospective studies would provide a higher level of evidence for testing causal hypotheses regarding the association of antiretroviral therapy with increased blood pressure. Future studies also need to investigate the phenotypes on the HIV clinical spectrum that are most susceptible to the effects of antiretroviral therapy on blood pressure and other cardio-metabolic parameters, as well as the efficacies of targeted therapies on these phenotypes.

REFLECTIONS: The Gibbs Model of Reflection (Gibbs, 1988).

(I). Description (*what happened?*)

I was admitted into the PhD program at the Warwick Medical School, University of Warwick in October 2013. In fulfillment of the requirements of the PhD degree award at the University, I was required to pass through an upgrading process from Master of Philosophy (MPhil) to PhD candidate within nine months of commencing the PhD program. The upgrade entailed submitting a 10,000-word document and an oral examination. My upgrade document comprised a systematic review with meta-analyses of studies regarding my chosen topic and a fully developed protocol for a primary research. The research protocol also had to be approved by the Biomedical & Scientific Research Ethics Committee (BSREC) of Warwick Medical School in order to progress beyond the upgrade process. The data for my primary research were to be collected from patients recruited at the Benue State University Teaching Hospital (BSUTH) in Nigeria. Hence, after passing my upgrade, I also obtained ethical approval from the BSUTH Health Research Ethics Committee. Thereafter, I began planning my trip to Nigeria. Before travelling, my supervisors and I had discussed several times with the Chief Medical Director of the teaching hospital concerning the logistics of the research project. Section 16.9 of Chapter 16 in my dissertation is a detailed account of the fieldwork (data collection) process in Nigeria. After completing my data collection, I returned to the University of Warwick to analyse the data. I commenced data analysis early on in my second year of PhD studies. I wrote up the analyses for journal publication during my second year and early on in my third year, as agreed with my supervisors. I had also led and submitted a series of systematic reviews and meta-analyses for journal publication during my second year. Most of the articles were accepted for publication. A few of them are currently under review. I now had enough materials and clarity to begin writing-up my PhD dissertation early on in

my third year. Within three months of commencing the write-up, I had completed and submitted the first draft to my supervisors. With mostly minor comments regarding the format of presentation, my dissertation was ready for submission much earlier than anticipated.

(II). Feelings (*what were you thinking and feeling?*)

Before commencing the programme, I was aware that the (full-time) PhD journey was not going to be an easy road to travel. Nonetheless, I felt quite optimistic about producing good work. I felt motivated to make the most during my time as a PhD student, especially regarding publications in peer-reviewed journals. There were concerns about travelling to Benue state in Nigeria for the field work, given that I had never been to that region of the country. However, I was quite confident in the background work my supervisors and I had done prior to the field work. The write-up of my PhD dissertation — as I assume it is the same for most PhD students —was an iterative process that involved revising the conceptual framework and re-analysing the data. Going back and forth, especially at this stage, was stressful, which occasionally made me feel a little less motivated. However, I always felt better after being reminded by my supervisors and my immediate family of the progress I had made so far.

(III). Evaluation (*what was good and bad about the experience?*)

No doubt, the process of obtaining a PhD is a rigorous one. However, I have acquired an immense set of skills as a researcher throughout this process — skills that have yielded evidence to advance the fields of HIV therapy and management. It was a great experience whenever an article of mine was accepted for publication. Of note, I had the best supervisors in the world. They offered me their support and technical expertise with great patience, and

allowed me to make decisions on how I wanted to be trained.

(IV). Analysis (*what sense can you make of the situation?*)

Throughout this process, I realise that I am a quick learner, and that most problems (if not all) have solutions. I also find that I am able to thrive in a career as a research epidemiologist, either in the academia or in industry.

(V). Conclusion (*what else could you have done?*)

Time went by rather quickly. However, I had sufficient time to complete my dissertation with publications in high-impact journals. I also attended several training workshops and seminars. To the best of my knowledge, I cannot think of anything that I would have done differently within the scope of my doctoral training.

(VI). Project (*if it arose again, what would you do?*)

I would probably want to conduct a randomized controlled trial to determine the clinical and cost-effectiveness of a new intervention targeting specific phenotypes on the HIV clinical spectrum to reduce susceptibility to the effects of antiretroviral therapy on cardio-metabolic disorders.

REFERENCES

Abebe M, Kinde S, Belay G, *et al*. (2014). Antiretroviral treatment associated hyperglycaemia and dyslipidaemia among HIV infected patients at Burayu health centre, Addis Ababa, Ethiopia: a cross-sectional comparative study. *BMC Res Notes*; 7: 380.

Aboud M, Elgalib A, Pomeroy L, *et al*. (2010). Cardiovascular risk evaluation and antiretroviral therapy effects in an HIV cohort: implications for clinical management: the CREATE 1 study. *Int J Clin Pract*; 64: 1252-1259.

Abrahams Z, Dave JA, Maartens G, Levitt NS (2015). Changes in blood pressure, glucose levels, insulin secretion and anthropometry after long term exposure to antiretroviral therapy in South African women. *AIDS Res Ther*; 12: 24; doi: 10.1186/s12981-015-0065-8.

Adewole OO, Eze S, Betiku Y, *et al*. (2010). Lipid profile in HIV/AIDS patients in Nigeria. *Afr. Health Sci*; 10: 144-149.

Adult Treatment Panel III (2002). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Final Report. *Circulation*; 106: 3143-3421.

Ahoua L, Umutoni C, Huerga H, *et al*. (2011). Nutrition outcomes of HIV-infected malnourished adults treated with ready-to use therapeutic food in sub-Saharan Africa: a longitudinal study. *J Int AIDS Society*, 14: 2 doi:10.1186/1758-2652-14-2.

AIDS Info (2009). HIV treatment failure. (URL https://aidsinfo.nih.gov/contentfiles/hivtreatmentregimenfailure_fs_en.pdf). Accessed 22 February 2016.

Ajetunmobi O (2002). Making sense of critical appraisal. Hodder Arnold: London.

Akhbar-Williams T (2010). Class Structure. In Smith JC. *Encyclopedia of African American Popular Culture*; 1: 322.

Akinboro AO, Onayemi O, Ayodele OE, *et al*. (2013). The impacts of first line highly active antiretroviral therapy on serum selenium, CD4 count and body mass index: a cross sectional and short prospective study. *Pan Afr Med J*; 15: 97.

Allavena C, Guimard T, Billaud E, *et al*. (2014). Prevalence and risk factors of sleep disturbances in a large HIV-infected adult population. *J Int AIDS Soc*; 17: 19576.

Allison P (2014). Prediction vs. causation in regression analysis. (URL http://statisticalhorizons.com/prediction-vs-causation-in-regression-analysis). Accessed 20 February 2016.

Ambrose JA, Barua RS (2004). The pathophysiology of cigarette smoking and cardiovascular disease: an update. *J Am Coll Cardiol*; 43: 1731-1737.

Arruda Junior ER, Lacerda HR, Vilela Moura LCR, *et al*. (2010a). Profile of Patients with Hypertension Included in a Cohort with HIV/AIDS in the State of Pernambuco, Brazil. *Arq Bras Cardiol*; 95: 640-647.

Arruda Junior ER, Lacerda HR, Vilela Moura LCR, *et al*. (2010b). Risk factors related to hypertension among patients in a cohort living with HIV/AIDS. *Braz J Infect Dis*; 14: 281-287.

Austin PC (2009). Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. *Statistics in Medicine*; 28: 3083-3107.

Austin PC (2011a). An introduction to propensity score methods for reducing the effects of

confounding in observational studies. *Multivariate Behav Res*; 46: 399-424.

Austin PC (2011b). A tutorial and case study in propensity score analysis: an application to estimating the effect of in-hospital smoking cessation counselling on mortality. *Multivariate Behav Res*; 46: 119-151.

Austin PC, Grootendorst P, Anderson GM (2007). A comparison of the ability of different propensity score models to balance measured variables between treated and untreated subjects: A Monte Carlo study. *Statistics in Medicine*; 26: 734-753.

Austin PC, Mamdani MM (2006). A comparison of propensity score methods: a case study estimating the effectiveness of post-AMI statin use. *Statistics in Medicine*; 25: 2084-2106.

Awotedu K, Ekpebegh C, Longo-Mbenza B, *et al*. (2010). Prevalence of metabolic syndrome assessed by IDF and NCEP ATP 111 criteria and determinants of insulin resistance among HIV patients in the Eastern Cape Province of South Africa. *Diabetes Metab Syndr: Clin Res Rev*; 4: 210-214.

Awotedu KO, Mbeza BL, Awotedu AA, Ekpebegh C (2015). Arterial stiffness in HIV patients in a semi-urban area of South Africa. *Clin Microbiol*; 4: 207.

Ayodele OE, Akinboro AO, Akinyemi SO, *et al*. (2013). Prevalence of Traditional Cardiovascular Risk Factors and Evaluation of Cardiovascular Risk Using Three Risk Equations in Nigerians Living with Human Immunodeficiency Virus. *N Am J Med Sci*; 5: 680-688.

Babaro G, Lacobellis G (2009). Metabolic syndrome associated with HIV and highly active antiretroviral therapy. *Curr Diab Rep*; 9: 37-42.

Baekken M, Os I, Sandvik L, Oektedalen O, *et al*. (2008). Hypertension in an urban HIV-positive population compared with the general population: influence of combination

antiretroviral therapy. *J Hypertens*; 26: 2126-2133.

Baekkeskov S, Aanstoot HJ, Christgau S, *et al.* (1990). Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature*; 347: 151–156.

Bajaj S, Tyagi SK, Bhargava A (2013). Metabolic syndrome in human immunodeficiency virus positive patients. *Ind J Endocrinol Metab*; 17: 117-120.

Bakare-Odunola MT, Enemaili I, Garba M, *et al*. (2008). The influence of lamivudine, stavudine and nevirapine on the pharmacokinetics of chlorpropamide in human subjects. *Eur J Drug Metab Pharmacokinet*; 33: 165-171.

Balasubramanyam A, Coraza I, Smith EO, *et al*. (2011). Combination of niacin and fenofibrate with lifestyle changes improves dyslipidaemia and hypoadiponectinaemia in HIV patients on antiretroviral therapy: results of "heart positive": a randomized controlled trial. *J Clin Endocrinol Metab*; 96: 2236-2247.

Barbaro G (2003). HIV infection, highly active antiretroviral therapy and the cardiovascular system. *Cardiovas Res*; 60: 87-95.

Barbaro G, Iacobellis G (2009). Metabolic syndrome associated with HIV and highly active antiretroviral therapy. *Curr Diab Rep*; 9: 37-42.

Baril J G, Kovacs CM, Trottier M, *et al*. (2007). Effectiveness and tolerability of oral administration of low-dose salmon oil to HIV patients with HAART-associated dyslipidemia. *HIV Clin Trials*; 8: 400-411.

Baron RM, Kenny DA (1986). The moderator-mediator variable distinction in social-psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol*; 51: 1173-1182.

Barve S, Kapoor R, Moghe A, *et al*. (2010). Focus on the liver: alcohol use, highly active antiretroviral therapy, and liver disease in HIV-infected patients. *Alcohol Res Health*; 33: 229-236.

Batista JL, Militão de Albuquerque Mde F, Ximenes RA, *et al*. (2013). Prevalence and socioeconomic factors associated with smoking in people living with HIV by sex, in Recife, Brazil. *Braz J Epidemiol*; 16: 432-443.

Baum MK, Rafie C, Lai S, *et al*. (2010). Alcohol Use Accelerates HIV Disease Progression. *AIDS Res Hum Retroviruses*; 25: 511-518.

Bello-Mojeed MA, Omigbodun OO, Ogun OC, *et al*. (2013). The relationship between the pattern of impairments in autism spectrum disorder and maternal psychosocial burden of care. *OA Autism*; 1: 4.

Benowitz NL (2003). Cigarette smoking and cardiovascular disease: Pathophysiology and implications for treatment. *Prog Cardiovasc Dis*; 46: 91-111.

Bergersen BM, Sandvik L, Bruun JN, Tonstad S (2004). Elevated Framingham risk score in HIV-positive patients on highly active retroviral therapy: results from Norwegian study of 721 subjects. *Eur J Clin Microbiol Infect Dis*; 23: 625-630.

Bergersen BM, Sandvik L, Dunlop O, *et al.* (2003). Prevalence of hypertension in HIV-positive patients on Highly Active Anti-retroviral Therapy (HAART) compared with HAART-naïve and HIV-negative controls: results from a Norwegian study of 721 patients. *Eur J Clin Microbiol Infect Dis*; 22: 731-736.

Bergersen BM, Schumacher A, Sandvik L, *et al*. (2006). Important differences in components of the metabolic syndrome between HIV-patients with and without highly active antiretroviral therapy and healthy controls. *Scan. J Infect Dis*; 38: 682-689.

Blann AD, Lip GYH, Islim IF, *et al*. (1997). Evidence of platelet activation in hypertension. *J Hum Hypertens*; 11: 607-609.

Blashill AJ, Mayer KH, Crane H, *et al*. (2013). Physical activity and health outcomes among HIV-infected men who have sex with men: a longitudinal mediational analysis. *Ann Behav Med*; 46: 149-156.

Blass SC, Ellinger S, Vogel M, *et al*. (2008). Overweight HIV patients with abdominal fat distribution treated with protease inhibitors are at high risk for abnormalities in glucose metabolism – a reason for glycaemic control. *Eur J Med Res*; 13: 209-214.

Blaton VH, Korita I, Bulo A (2008). How is metabolic syndrome related to dyslipidaemia? *Biochem Medica*; 18: 14-24.

Bloomfield GS, Hogan JW, Keter A (2011). Hypertension and obesity as cardiovascular risk factors among HIV seropositive patients in western Kenya. *PLoS ONE*; 6: e22288.

Bloomfield GS, Velazquez EJ (2013). HIV and cardiovascular disease in sub-Saharan Africa. *J Am Coll Cardiol*; 61: 2391-2396.

Blümer RM, van Vonderen MG, Sutinen J, *et al*. (2008). Zidovudine/lamivudine contributes to insulin resistance within 3 months of starting combination antiretroviral therapy. *AIDS*; 22: 227-236.

Bonaa KH, Thelle DS (1991). Association between blood pressure and serum lipids in a population: the Tromso study. *Circulation*; 83: 1305-1314.

Bonacini M (2011). Alcohol use among patients with HIV infection. *Ann Hepatol*; 10: 502-507.

Bonfanti P, Giannattasio C, Ricci E, *et al*. (2007). HIV and metabolic syndrome: a comparison with the general population. *J Acquir Immune Defic Syndr*; 45: 426-431.

Bonfanti P, De Socio GV, Ricci E, *et al*. (2012). The feature of metabolic syndrome in HIV-naïve patients is not the same of those treated: results from a prospective study. *Biomed Pharmacother*; 66: 348-353.

Borenstein M, Hedges L, Rothstein H (2007). Meta-analysis fixed effect vs. random effects. (URL https://www.meta-analysis.com/downloads/Meta-analysis%20fixed%20effect%20vs%20random%20effects.pdf). Accessed 15 February 2016.

Bosamiya SS (2011). The immune reconstitution inflammatory syndrome. *Indian J Dermatol*; 56: 476-479.

Bowers D, House A, Owens D (2011). Getting started in health research. Blackwell: Sussex

Brown MB, Forsythe AB (1974). The small-sample behaviour of some statistics which test the equality of several means. *Technometrics*; 16: 129-132.

Brown TT, Cole SR, Kingsley LA, *et al*. (2005). Antiretroviral Therapy and the prevalence and incidence of diabetes in a multicentre AIDS Cohort study. *Arch Intern Med*; 165: 1179-1184.

Bruno RM, Palagini L, Gemignani A, *et al*. (2013). Poor sleep quality and resistant hypertension. *Sleep Med*; 14: 1157-1163.

Buchacz K, Weidle PJ, Moore D, *et al*. (2008). Changes in lipid profile over 24 months among adults on first-line highly active antiretroviral therapy in the home-based aids care program in rural Uganda. *J Acquir Immune Defic Syndr*; 47: 304-311.

Buck C (1975). Popper's philosophy for epidemiologists. *Int J Epidemiol*; 4: 159-168.

Buysse DJ, Reynolds CF, Monk TH, *et al*. (1989). The Pittsburgh Sleep Quality Index (PSQI): A new instrument for psychiatric research and practice. *Psychiatry Research*; 28: 193-213.

Calvo M, Laguno M, Martínez M, Martínez E (2015). Effects of Tobacco Smoking on HIV-Infected Individuals. *AIDS Rev*; 17: 47-55.

Calza L, Manfredi R, Chiodo F (2003). Statins and fibrates for the treatment of hyperlipidaemia in HIV-infected patients receiving HAART. *AIDS*; 17: 851-859.

Calza L, Manfredi R, Colangeli V. *et al*. (2005). Substitution of nevirapine or efavirenz for protease inhibitor versus lipid- lowering therapy for the management of dyslipidaemia. *AIDS*; 19: 1051-1058.

Calza L, Masetti G, Piergentili B, *et al*. (2011). Prevalence of diabetes mellitus, hyperinsulinaemia and metabolic syndrome among 755 adult patients with HIV-1 infection. *Int J STD AIDS*; 22: 43-45.

Carey D, Amin J, Boyd M, *et al*. (2010). Lipid profiles in HIV-infected adults receiving atazanavir and atazanavir/ritonavir: systematic review and meta-analysis of randomized controlled trials. *J Antimicrob Chemother*; 65: 1878-1888.

Carey RAB, Rupali P, Abraham OC, Kattula D (2013). Does first line antiretroviral therapy increase the prevalence of cardiovascular risk factors in Indian patients? A cross-sectional study. *J Postgrad Med*; 59: 258-262.

Carr A, Cooper DA (2000). Adverse effects of antiretroviral therapy. *Lancet*; 356: 1423-1430.

Carr A, Samaras K, Chisholm DJ, Cooper DA (1998). Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance.

Lancet; 351: 1881-1883.

Ceccato MGB, Bonolo PF, Souza AI, *et al*. (2011). Antiretroviral therapy-associated dyslipidaemia in patients from a reference centre in Brazil. *Braz J Med Biol Res*; 44: 1177-1183.

Celermajer DS, Sorensen KE, Georgakopoulos D, *et al*. (1993). Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation*; 88: 2149-2155.

Centre for Reviews and Dissemination (2008). Systematic Reviews: CRD's Guidance for Undertaking Systematic Reviews in Health Care. (URL http://www.york.ac.uk/inst/crd/pdf/Systematic_Reviews.pdf). Accessed 20 August 2014.

Chander G, Lau B, Moore R (2006). Hazardous alcohol use: a risk factor for nonadherence and lack of suppression in HIV infection. *J Acquir Immune Defic Syndr*; 43: 411-417.

Chow DC, Souza SA, Chen R, *et al*. (2003). Elevated blood pressure in HIV-infected individuals receiving highly active antiretroviral therapy. *HIV Clin Trials*; 4: 411-416.

Cochran WG (1965). The planning of observational studies of human populations (with discussion) *Journal of the Royal Statistical Society*; 128: 134–155.

Cohen J (1988). Statistical power analysis for the behavioural sciences. Erlbaum: New Jersey.

Cohen J, Cohen P, West SG, Aiken LS (2003). Applied multiple regression/correlation analysis for the behavioural sciences. Erlbaum: New Jersey.

Cooper CL, Cameron DW (2005). Effect of alcohol use and highly active antiretroviral therapy on plasma levels of hepatitis C virus (HCV) in patients co-infected with HIV and

HCV. *Clin Infect Dis*; 41: 105-109.

Cox LA (2012). Improving risk analysis. Springer: New York.

Crothers K, Griffith TA, McGinnis KA, *et al*. (2005). The impact of cigarette smoking on mortality, quality of life, and comorbid illness among HIV-positive veterans. *J Gen Intern Med*; 20: 1142-1145.

Crum-Cianflone NF, Roediger M, Eberly LE, *et al*. (2010). Obesity among HIV-infected persons: impact of weight on CD4 cell count. *AIDS*; 24: 1069-1072.

Currier (2015). Epidemiology of cardiovascular disease and risk factors in HIV-infected patients. (URL http://www.uptodate.com/contents/epidemiology-of-cardiovascular-disease-and-risk-factors-in-hiv-infected-patients). Accessed 24 March 2016.

Daar ES, Tierney C, Fischl MA, *et al*. (2011). Atazanavir plus ritonavir or efavirenz as part of a 3-drug regimen for initial treatment of HIV-1. *Ann Intern Med*; 154*:* 445-456

Dagogo-Jack S (2008). HIV therapy and diabetes risk. *Diabetes Care*; 31: 1267-1268.

Daly MC, Duncan GJ, McDonough P, Williams DR (2002). Optimal indicators of socioeconomic status for health research. *Am J Public Health*; 92: 1151-1157.

Data Collection on Adverse Events of Anti-HIV Drugs Study Group (2007). Class of antiretroviral drugs and the risk of myocardial infarction. *N Engl J Med*; 356: 1723-1735.

Dau B, Holodniy M (2008). The relationship between HIV infection and cardiovascular disease. *Curr Cardiol Rev*; 4: 203-218.

Deeks SG, Kar S, Gubernick SI, Kirkpatrick P (2008). Fresh from the pipeline: raltegravir. *Nat Rev*; 7: 117-118.

Delgado-Rodriguez M, Llorca J (2004). Bias. *J Epidemiol Community Health*; 58: 635-641.

Denue A, Muazu J, Gashau W, *et al*. (2012). Effects of highly active antiretroviral therapy (HAART) on blood pressure changes and its associated factors in HAART naïve HIV-infected patients in North-Eastern Nigeria. *Arch Appl Sci Res*; 4: 1447-1452.

Denue BA, Ikunaiye PNY, Denue CBA (2013). Body mass index changes during highly active antiretroviral therapy in Nigeria. *East Mediterr Health J*; 19: S89-S97.

Denue BA, Alkali MB, Abjah AU, *et al*. (2013). Changes in lipid profiles and other biochemical parameters in HIV-1 infected patients newly commenced on HAART regimen. *Infect Dis (Auckl)*; 6: 7-14.

DerSimonian R, Laird N (1986). Meta-analysis in clinical trials. *Control Clin Trials*; 7:177-188.

Dillon DG, Gurdasani D, Riha J, *et al*. (2013). Association of HIV and ART with cardiometabolic traits in sub-Saharan Africa: a systematic review and meta-analysis. *Int J Epidemiol*; 42: 1754-1771.

Dimala CA, Atashili J, Mbuagbaw JC, *et al*. (2016). Prevalence of Hypertension in HIV/AIDS Patients on Highly Active Antiretroviral Therapy (HAART) Compared with HAART-Naïve Patients at the Limbe Regional Hospital, Cameroon. *PLoS One*; 11: e0148100.

Dimodi HT, Etame LS, Nguimkeng BS (2014). Prevalence of Metabolic Syndrome in HIV-Infected Cameroonian Patients. *World J AIDS*; 4: 85-92.

Dirajlal-Fargo S, Webel AR, Longenecker CT, *et al*. (2015). The effect of physical activity on cardio-metabolic health and inflammation in treated HIV infection. *Antivir Ther*; doi:10.3851/imp2998.

Djukpen RO (2012). The geography of HIV/AIDS and an assessment of risk factor perspectives in Nigeria: the case of Benin City and Makurdi. (URL https://www.ideals.illinois.edu/handle/2142/42192). (Accessed 01 July 2014).

Domingos H, Cunha RV, Paniago AM, *et al*. (2009). Metabolic effects associated to the highly active antiretroviral therapy (HAART) in AIDS patients. *Braz J Infect Dis*; 13: 130-136.

Donati KG, Cauda R, Lacoviello L (2010). HIV Infection, Antiretroviral Therapy and Cardiovascular Risk. *Mediterr J Hematol Infect Dis*; 2: e2010034.

Dong-Qing Z, Chang-Quan H, Yan-Ling Z, *et al*. (2014). Cigarette smoking is associated with increased diastolic blood pressure among Chinese nonagenarians/centenarians. *Blood Press*; 23: 168-173.

Donini LM, Poggiogalle E, del Balzo V, *et al*. (2003). How to estimate fat mass in overweight and obese subjects. *Int J Endocrinol*; 2013: 285680.

Dube M, Fenton M (2003). Lipid abnormalities. *Clin Infect Dis*; 36: S79-S83.

Dube MP, Sattler FR (1998). Metabolic complications of antiretroviral therapies. *AIDS Clin Care*; 10: 41-44.

Dubé MP, Stein JH, Aberg JA, *et al*. (2003). Guidelines for the evaluation and management of dyslipidemia in human immunodeficiency virus (HIV)-infected adults receiving antiretroviral therapy: recommendations of the HIV Medical Association of the Infectious Disease Society of America and the Adult AIDS Clinical Trials Group. *Clin Infect Dis*; 37: 613-627.

Duncan GJ, Daly MC, McDonough P, Williams DR (2002). Optimal Indicators of Socioeconomic Status for Health Research. *Am J Public Health*; 92: 1151-1157.

Duval S, Tweedie R (2000). A non-parametric "Trim and Fill" method of accounting for publication bias in meta-analysis. *J Am Stat Assoc*; 95: 89-98.

Edwards JK, Cole SR, Westreich D, *et al*. (2015). Age at entry into care, timing of antiretroviral therapy initiation, and 10-year mortality among HIV-seropositive adults in the United States. *Clin Infect Dis*; 61: 1189-1195.

Egger M, Smith GD, Schneider M, Minder C (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*; 315: 629.

Eira M, Bensenor IM, Dorea EL, *et al*. (2012). Potent antiretroviral therapy for human immunodeficiency virus infection increases aortic stiffness. *Arq Bras Cardiol*; 99: 1100-1107.

Ekali LG, Johnstone LK, Echouffo-Tcheugui JB, *et al*. (2013). Fasting blood glucose and insulin sensitivity are unaffected by HAART duration in Cameroonians receiving first-line antiretroviral treatment. *Diabet & Metab*; 39: 71-77.

El-Sadr WM, Mullin CM, A Carr A, *et al*. (2005) Effects of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naïve cohort. *HIV Med*; 6: 114-121.

Esposito FM, Coutsoudis A, Visser J, Kindra G (2008). Changes in body composition and other anthropometric measures of female subjects on highly active antiretroviral therapy (HAART): a pilot study in Kwazulu-Natal, South Africa. *S Afr J HIV Med*; 9: 36-42.

Federal Ministry of Health Nigeria (2012). National guidelines for HIV and AIDS treatment and care in adolescents and adults. (URL http://www.who/guidelines/nigeria_art.pdf). Accessed April 23 2015.

Feldman JG, Minkoff H, Schneider MF, *et al*. (2006). Association of cigarette smoking with HIV prognosis among women in the HAART era: a report from the women's interagency

HIV study. *Am J Public Health*; 96: 1060-1065.

Feleke Y, Fekade D & Mezegebu Y (2012). Prevalence of highly active antiretroviral therapy associated metabolic abnormalities and lipodystrophy in HIV infected patients. *Ethiopian Medical Journal*, 50: 221-230.

Field A (2012). Discovering statistics: one-way independent ANOVA. (URL http://www.statisticshell.com/docs/onewayanova.pdf). Accessed 20 February 2016.

Fitch KV, Anderson EJ, Hubbard JL, *et al*. (2006). Effects of a lifestyle modification program in HIV-infected patients with the metabolic syndrome. *AIDS*; 20: 1843-1850.

Fleischman A, Johnsen S, Systrom DM, *et al*. (2007). Effects of a nucleoside reverse transcriptase inhibitor, stavudine, on glucose disposal and mitochondrial function in muscle of healthy adults. *Am J Physiol Endocrinol Metab*; 292: e1666-e1673.

Florindo AA, de Oliveira Latorre Mdo R, Jaime PC, Segurado AA (2007). Leisure time physical activity prevents accumulation of central fat in HIV/AIDS subjects on highly active antiretroviral therapy. *Int J STD AIDS*; 18: 692-696.

Fontas E, van Leth F, Sabin CA, *et al*. (2004). Lipid profiles in HIV-infected patients receiving combination antiretroviral therapy: are different antiretroviral drugs associated with different lipid profiles? *J Infect Dis*; 189: 1056–1074.

Friis-Moller N, Weber R, Reiss P, *et al*. (2003). Cardiovascular disease risk factors in HIV patients –association with antiretroviral therapy. Results from the DAD study. *AIDS*; 17: 1179-1193.

Friis-Møller N, Thiébaut R, Reiss P, *et al*. (2010). Predicting the risk of cardiovascular disease in HIV-infected patients: the Data Collecton on Adverse Effects of Anti-HIV Drugs

Study. *Eur J Cardiovasc Prevent Rehab*; 17: 491-501.

Fritz MS, MacKinnon DP (2007). Required sample size to detect the mediated effect. *Psychol Sci*; 18: 233-239.

Garawi F, Devries K, Thorogood N, Uauy R (2014). Global differences between women and men in the prevalence of obesity: is there an association with gender inequality? *Eur J Clin Nutr*; 68: 1101-1106.

Garver MS, Mentzer JT (1999). Logistics research methods. Employing structural equation modeling to test for construct validity. *J Bus Logist*; 20: 33-57.

Gedefaw L, Yemane T, Sahlemariam Z, Yilma D, *et al*. (2013). Anaemia and risk factors in HAART naive and HAART experienced HIV positive persons in South West Ethiopia: A comparative study. *PLoS One*; 8: e72202.

George D, Mallery M (2010). SPSS for Windows Step by Step: A Simple Guide and Reference, 17.0 update. Pearson: Boston.

Gibbs G. (1988) *Learning by doing: A guide to teaching and learning methods*. Oxford: University of Oxford Polytechnic.

Gilliam LK, Palmer JP, Lernmark Å (2004). Autoantibodies and the disease process of type 1 diabetes mellitus. *In* LeRoith D, Taylor SI, Olefsky JM (editors); *Diabetes Mellitus: A Fundamental and Clinical Text*, 3rd ed. Lippincott: Philadelphia.

Glass TR, Ungsedhapand C, Wolbers M, *et al*. (2006). Prevalence of risk factors for cardiovascular disease in HIV-infected patients over time: the Swiss HIV Cohort Study. *HIV Med*; 7*:* 404-410.

Glover J (2007). Riven by class and no social mobility - Britain in 2007. The Guardian: London.

Goedecke JH, Micklesfield LK, Levitt NS, *et al*. (2013). Effect of different antiretroviral drug regimens on body fat distribution of HIV-infected South African women. *AIDS Res Hum Retroviruses*; 29: 557-563.

Gowdiah PK, Neminatha RE, Channappa MU, *et al*. (2013). Plasma lipids and highly active antiretroviral therapy; a prospective study. *Ind J Sci Res and Tech*; 1: 53-61.

Grandominico JM, Fichtenbaum CJ (2008). Short-term effect of HAART on blood pressure in HIV-infected individuals. *HIV Clin Trials*; 9: 52-60.

Grilli L, Rampichini C (2011). Propensity scores for the estimation of average treatment effects in observational studies. (URL http://www.bristol.ac.uk/media-library/sites/cmm/migrated/documents/prop-scores.pdf). Accessed 20 May 2015.

Grinspoon S (2009). Diabetes Mellitus, Cardiovascular Risk, and HIV Disease. *Circulation*; 119: 770-772.

Grossman E, Messerli FH (2004). Iatrogenic and drug-induced hypertension. In Mansoor GA (editor), *Secondary Hypertension: Clinical Presentation, Diagnosis, and Treatment* (Chapter 2, pp. 21-35). Human Press Inc: New Jersey.

Grunfeld C, Kotler DP, Shigenaga JK, *et al*. (1991). Circulating interferon-alpha levels and hypertriglyceridemia in the acquired immunodeficiency syndrome. *Am J Med*; 90: 154.

Grunfeld C, Pang M, Doerrler W, *et al*. (1992). Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *J Clin Endocrinol Metab*; 74: 1045.

Grunfeld C, Saag M, Cofrancesco J, *et al*. (2010). Regional adipose tissue measured by MRI over 5 years in HIV-infected and control participants indicates persistence of HIV-associated lipoatrophy. *AIDS*; 24: 1717-1726.

Gunzler D, Chen T, Wu P, Zhang H (2013). Introduction to mediation analysis with structural equation modeling. *Shanghai Arch Psychiatry*; 25: 390-394.

Haavelmo T (1943). The statistical implications of a system of simultaneous equations. *Econometrica*; 11: 1–12.

Hansen BR, Petersen J, Haugaard SB, *et al*. (2009). The prevalence of metabolic syndrome in Danish patients with HIV infection: the effect of antiretroviral therapy. *HIV Med*; 10: 378-387.

Harbord RM, Harris RJ, Sterne JAC (2009). Updated tests for small-study effects in meta-analyses. The Stata Journal; 9: 197-210.

Harrison C, Pompilius M, Pinkerton K, Ballinger S (2011). Mitochondrial oxidative stress significantly influences atherogenic risk and cytokine-induced oxidant production. *Environ Health Perspect*; 119: 676-681.

Hartzell JD, Janke IE, Weintrob AC (2008). Impact of depression on HIV outcomes in the HAART era. *J Antimicrob Chemother*; 62: 246-255.

Hasse B, Iff M, Ledergerber B, *et al*. (2014). Obesity trends and body mass index changes after starting antiretroviral treatment: The Swiss HIV cohort study. *Open Forum Infect Dis*; 1: doi:10.1093/ofid/ofu040.

Hemingway H, Marmot M (1999). Psychosocial factors in the aetiology and prognosis of coronary heart disease: systematic review of prospective cohort studies. *BMJ*; 318: 1460-1467.

Hemingway P, Brereton N (2009). What is a systematic review? Evidence-based medicine. (URL http://www.medicine.ox.ac.uk/bandolier/painres/download/whatis/syst-review.pdf). Accessed 4 March 2015.

Henneken CH, Buring JE (1987). Epidemiology in Medicine. Little, Brown and Company: Boston.

Higgins JP, Thompson SG (2002). Quantifying heterogeneity in a meta-analysis. *Stat Med*; 21: 1539-1558.

Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003). Measuring inconsistency in meta-analyses. *BMJ*; 327: 557-560.

Higgins JPT, Green S (2011). Cochrane Handbook for Systematic Reviews of Interventions. (URL www.cochrane-handbook.org). Accessed 20 December 2014.

Hill BA (1965). The environment and disease: association or causation? *Proc R Soc Med*; 58: 295-300.

Hjermann I, Helgeland A, Holme I, *et al*. (1978). The association between blood pressure and serum cholesterol in healthy men: the Oslo study. *J Epidemiol Comm Health*; 32: 117-123.

Hoelter DR (1983). The analysis of covariance structures: goodness-of-fit indices. *Socio Meth Res*; 11: 325-344.

Hoevenaar-Blom MP, Spijkerman AM, Kromhout D, *et al*. (2011). Sleep duration and sleep quality in relation to 12-year cardiovascular disease incidence: the MORGEN study. *Sleep*; 34: 1487-1492.

Hooshyar D, Hanson DL, Wolfe M, *et al*. (2007). Trends in perimortal conditions and mortality rates among HIV-infected patients. *AIDS*; 21: 2093-2100.

Howard E, Mullen A, Stradling C. (2014). A metabolic syndrome among HIV infected outpatients in the era of highly active antiretroviral therapy: prevalence and contributing

factors. *European J Nutr Food Saf*; 4: 281-282.

Hughes PJ, Cretton-Scott E, Teague A, Wensel TM (2011). Protease inhibitors for patients with HIV-1 infection: a comparative overview. *Phys Ther*; 36: 332-345.

Hume D (2000). A treatise of human nature. Oxford University Press: Oxford and New York.

Hyman HH (1955). Survey design and analysis. Glencoe: New York.

Imbens GW (2004). Nonparametric estimation of average treatment effects under exogeneity: A review. *The Review of Economics and Statistics*; 86: 4-29.

Institute of Alcohol Studies (2013). A good measure: units and drinking guidelines. (URL http://www.ias.org.uk/Alcohol-knowledge-centre/Consumption/Factsheets/A-good-measure-Units-and-drinking-guidelines.aspx). Accessed 26 December 2013.

International Centre for Alcohol Policies (2010). International drinking guidelines. (URL http://www.icap.org/table/Internationaldrinkingguidelines). Accessed 26 December 2013.

International Diabetes Federation (2006). The IDF consensus worldwide definition of the metabolic syndrome. (URL http://www.idf.org/webdata/docs/MetS_def_update2006.pdf). Accessed 16 August 2015.

Jaff NG, Norris SA, Snyman T, *et al*. (2015). Body composition in the Study of Women Entering and in Endocrine Transition (SWEET): A perspective of African women who have a high prevalence of obesity and HIV infection. *Metabolism*; 64: 1031-1041.

Jaime PC, Florindo AA, Latorre Mdo R, Segurado AA (2006). Central obesity and dietary intake in HIV/AIDS patients. *Revista de Saúde Pública*, 40: 634-640.

Jain N, Dandu H, Verma SP, *et al*. (2013). An observational study on the prevalence of

dyslipidaemia and dysglycaemia in human immunodeficiency virus patients. *Ann Trop Med Public Health*; 6: 84-88.

James LR, Brett JM (1984). Mediators, moderators and tests for mediation. *Journal of Applied Psychology*; 69: 307-321.

Jamison DT, Feachem RG, Makgoba MW, *et al*. (2006). Disease and Mortality in Sub-Saharan Africa. World Bank: Washington, DC.

Janssen I, Katzmarzyk PT, Ross R (2004). Waist circumference and not body mass index explains obesity-related health risk. *Am J Clin Nutr*; 79: 379-384.

Jantarapakde J, Phanuphak N, Chaturawit C, *et al*. (2014). Prevalence of metabolic syndrome among antiretroviral-naive and antiretroviral-experienced HIV-1 infected Thai adults. *AIDS Patient Care STDs*; 28: 331–340.

Jasjeet SS (2007). The Neyman-Rubin model of causal inference and estimation via matching methods. (URL http://sekhon.berkeley.edu/papers/SekhonOxfordHandbook.pdf). Accessed 14 may 2015.

Jericó C, Knobel H, Montero M, *et al*. (2005). Hypertension in HIV-infected patients: prevalence and related factors. *Am J Hypertens*; 18: 1396-1401.

Johnson HM, Gossett LK, Piper ME, *et al*. (2010). Effects of Smoking and Smoking Cessation on Endothelial Function: One-Year Outcomes from a Randomized Clinical Trial. *J Am Coll Cardiol*; 55: 1988-1995.

Joint United Nations Programme on HIV/AIDS & World Health Organization (2006). Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public approach. (URL http://www.who.int/hiv/pub/guidelines/artadultguidelines.pdf). Accessed 3 January 2014.

Joint United Nations Programme on HIV/AIDS (2013). Report on the global AIDS epidemic. (URL http://www.unaids.org/en/..../UNAIDS_Global_Report_2013_en.pdf). Accessed 4 November 2015.

Joint United Nations Programme on HIV/AIDS (2014a). Fact sheet 2014: Global statistics. (URL http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/factsheet/2014/20140716_FactSheet_en.pdf). Accessed 10 December 2015.

Joint United Nations Programme on HIV/AIDS (2014b) Press release: UNAIDS report shows that 19 million of the 35 million people living with HIV today do not know that they have the virus. (URL http://www.unaids.org/sites/default/files/web_story//20140716_PR_GapReport_en.pdf). Accessed 10 December 2015.

Joy T, Keogh HM, Hadigan C, *et al*. (2007). Dietary fat intake and relationship to serum lipid levels in HIV-infected patients with metabolic abnormalities in the HAART era. *AIDS*; 21: 1591-1600.

Joy T, Keogh HM, Hadigan C, *et al*. (2008). Relation of body composition to body mass index in HIV-infected patients with metabolic abnormalities. *J Acquir Immune Defic Syndr*; 47:174-184.

Joynt KE, Whellan DJ, O'Connor CM (2003). Depression and cardiovascular disease: mechanisms of interaction. *Biol Psychiatry*; 54: 248-261.

Judd CM, Kenny DA (1981). Process analysis: Estimating mediation in treatment evaluations. *Evaluation Review*; 5: 602-619.

Judd CM, Kenny DA (2010). Data analysis. In Gilbert D, Fiske ST, Lindzey G (Eds.), *The*

handbook of social psychology (5th ed., Vol. 1, pp. 115-139), New York.

Julius H, Basu D, Ricci E (2011). The burden of metabolic diseases amongst HIV positive patients on HAART attending the Johannesburg Hospital. *Curr HIV Res*; 9: 247-252.

Kagaruki GB, Mayige MT, Ngadaya ES, *et al*. (2014) Magnitude and risk factors of non-communicable diseases among people living with HIV in Tanzania: a cross sectional study from Mbeya and Dar es Salaam regions. *BMC Public Health*; 14: 904

Kalra S, Agrawal N (2013). Diabetes and HIV: current understanding and future perspectives. *Curr Diab Rep*; 13: 419-427.

Kalra S, Kalra B, Agrawal N, Unnikrishnan AG (2011). Understanding diabetes in patients with HIV/AIDS. *Diabetol Metab Syndr*; 3: 2.

Kamangar F (2012). Effect modification in epidemiology and medicine. *Arch Iran Med*; 15: 575-582.

Kamath A, Shivaprakash G, Adhikari P (2011). Body mass index and waist circumference in type 2 diabetes mellitus patients attending a diabetes clinic. *Int J Biol Med Res*; 2: 636-638.

Kaplan R, Kingsley LA, Sharrett AR, *et al*. (2007). Ten-Year Predicted Coronary Heart Disease Risk in HIV-Infected Men and Women. *Clin Infect Dis*; 45: 1074-1081.

Kaufman DL, Erlander MG, Clare-Salzler M, *et al.* (1992). Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. *J Clin Invest*; 89: 283-292.

Kaur J (2014). A comprehensive review on metabolic syndrome. *Cardiol Res Practice*; 2014: 943162.

Kearney PM, Whelton M, Reynolds K, *et al*. (2005). Global burden of hypertension: analysis

of worldwide data. *Lancet*; 365: 217-223.

Kelly P, Zulu I, Amadi B, *et al.* (2002). Morbidity and nutritional impairment in relation to CD4 count in a Zambian population with high HIV prevalence. *Acta Trop*; 83: 151-158.

Kenny DA (2015). Mediation. (URL http://davidakenny.net/cm/mediate.htm#CI). Accessed 22 February 2016.

Kiage JN, Heimburger DC, Nyirenda CK, *et al*. (2013). Cardio-metabolic risk factors among HIV patients on antiretroviral therapy. *Lipids Health Dis*; 12: 1–9.

Kinabo GD, Sprengers M, Msuya LJ, *et al*. (2013). Prevalence of Lipodystrophy in HIV-infected children in Tanzania on highly active antiretroviral therapy. *Paediatr Infect Dis J*; 32: 39-44.

Kingsley LA, Cuervo-Rojas J, Munoz A, *et al*. (2008). Subclinical coronary atherosclerosis, HIV infection and antiretroviral therapy: multi-centre AIDS cohort study. *AIDS*; 22: 1589-1599.

Klatsky A (1995). Blood pressure and alcohol intake. *In*: LBM B (editor); *Hypertension: Pathophysiology, Diagnosis, and Management*, pp. 2649-2667. Raven Press: New York.

Koethe JR, Jenkins CA, Lau B, *et al*. (2015). Rising obesity prevalence and weight gain among adults starting antiretroviral therapy in the United States and Canada. (URL http://www.ncbi.nlm.nih.gov/pubmed/26352511). Accessed 18 January 2016.

Koopmans T (1953). Identification problems in econometric model construction. In Hood W & Koopmans T (editors), Studies in Econometric Method (pp. 27-48). Wiley: New York.

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

Krishnan S, Schouten JT, Atkinson B, *et al*. (2012). Metabolic syndrome before and after initiation of antiretroviral therapy in treatment-naïve HIV-infected individuals. *J Acquir Immune Defic Syndr*; 61: 381-389.

Lai S, Bartlett J, Lai H, *et al*. (2009). Long-Term Combination Antiretroviral Therapy Is Associated with the Risk of Coronary Plaques in African Americans with HIV Infection. *AIDS Patient Care STDS*; 23: 815-824.

Laonnidis JP, Cappelleri JC, Lau J (1998). Issues in comparisons between meta-analyses and large trials. *JAMA*; 279: 1089-1093.

Larsson R, Capili B, Eckert-Norton M, *et al*. (2006). Disorders of glucose metabolism in the context of human immunodeficiency virus infection. *J Am Assoc Nurse Pract*; 18: 92-103.

Law MG, Friis-Møller N, El-Sadr WM, *et al*. (2006). The use of the Framingham equation to predict myocardial infarctions in HIV-infected patients: comparison with observed events in the DAD study. *HIV Med*; 7: 218-230.

Lekakis J, Ikonomidis I, Palios J, *et al*. (2009). Association of highly active antiretroviral therapy with increased arterial stiffness in patients infected with Human Immunodeficiency Virus. *Am J Hypertens*; 22: 828-834.

Levy AR, McCandless L, Harrigan PR, *et al*. (2005). Changes in lipids over twelve months after initiating protease inhibitor therapy among persons treated for HIV/AIDS. *Lipids Health Dis*; 4: 4.

Lin YJ, Yang CH, Huang YF, Lai AC (2011). The incidence and prevalence of chronic diseases among HIV-infected patients on highly active antiretroviral therapy (HAART). *Taiwan J Public Health*; 30: 549-557.

López S, Miró O, Martínez E, *et al*. (2004). Mitochondrial effects of antiretroviral therapies

in asymptomatic patients. *Antivir Ther*; 9: 47-55.

Lucas RM, McMichael AJ (2005). Association or causation: evaluating links between environment and disease. *Bull World Health Organ*; 83: 792-795.

Lyons A, Costagliola D, Mussini C, *et al*. (2015). Is HIV Infection Duration Associated with Myocardial Infarctions? *15th European AIDS Conference, Barcelona*. Abstract PS11/3.

MacCorquodale K, Meehl PE (1948). On a distinction between hypothetical constructs and intervening variables. *Psychological Review*; 55: 95-107.

MacKinnon D (2008). Introduction to Statistical Mediation Analysis. Lawrence Erlbaum Associates: New York.

MacKinnon DP, Lockwood CM, Williams J (2004). Confidence limits for the indirect effect: distribution of the product and resampling methods. *Multivariate Behav Res*; 39: 99-128.

Magenta L, Dell-Kuster S, Richter WO, *et al*. (2011). Lipid and lipoprotein profile in HIV-infected patients treated with Lopinavir/Ritonavir as a component of the first combination antiretroviral therapy. *AIDS Res Hum Retroviruses*; 27: 525-533.

Malapati B, Patel B, Shah RM, *et al*. (2014). Changes in lipid profile and other biochemical parameters in HIV-1 infected patients. *Int J Med Sci Public Health*; 3: 813–817.

Maloberti A, Giannattasio C, Dozio D, *et al*. (2013). Metabolic syndrome in human immunodeficiency virus-positive subjects: prevalence, phenotype, and related alterations in arterial structure and function. *Metab Syndr Relat Disord*; 11: 403-411.

Manner I, Baekken M, Oektedalen O, Os I (2012). Hypertension and antihypertensive treatment in HIV-infected individuals. A longitudinal cohort study. *Blood Press*; 21: 311–319.

Manner IW, Baekken M, Oektedalen O, *et al*. (2010). Effect of HIV duration on ambulatory blood pressure in HIV-infected individuals with high office blood pressure. *Blood Press*; 19: 188-195.

Manner IW, Trøseid M, Oektedalen O, *et al*. (2013). Low nadir CD4 cell count predicts sustained hypertension in HIV-infected individuals. *J Clin Hypertens (Greenwich)*; 15: 101-106.

Manuthu EM, Joshi MD, Lule GN, Karari E (2008). Prevalence of dyslipidaemia and dysglycaemia in HIV infected patients. *East Afr Med J*; 85: 10-17.

Mariz CA, Militão M, Ximenes RA, *et al*. (2011). Body mass index in individuals with HIV infection and factors associated with thinness and overweight/obesity. *Cad Saúde Pública*; 27: 1997-2008.

Market Research Society (2006). Occupation groupings: a job dictionary. MRS: London.

Marmot MG, Bosma H, Hemingway H, *et al*. (1997). Contribution of job control and other risk factors to social variations in coronary heart disease incidence. *Lancet*; 350: 235-239.

Masia M, Bernal, Padilla S, *et al*. (2009). A pilot randomized trial comparing an intensive versus a standard intervention in stable HIV-infected patients with moderate-high cardiovascular risk. *J Antimicrob Chemother.* doi:10.1093/jac/dkp250.

Mathers C, Stevens G, Mascarenhas M (2009). Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks. (URL http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf). Accessed 30 March 2016.

Mathers CD, Loncar D (2005). Updated projections of global mortality and burden of disease, 2002-2030: data sources, methods and results. (URL

http://www.who.int/healthinfo/statistics/bodprojectionspaper.pdf). Accessed 10 April 2014.

Mayo Clinic (2012). HDL cholesterol: How to boost your 'good' cholesterol. (URL http://www.mayoclinic.org/diseases-conditions/high-blood-cholesterol/in-depth/hdl-cholesterol/art-20046388). Accessed 29 March 2015.

Mbunkah HA, Meriki HD, Kukwah AT, *et al*. (2014). Prevalence of metabolic syndrome in human immunodeficiency virus-infected patients from the South-West region of Cameroon, using the adult treatment panel III criteria. *Diabetol Metab Syndr*; 6: 92.

Medina-Torne S, Ganesan A, Barahona I, Crum-Cianflone NF (2012). Hypertension is common among HIV-infected persons, but not associated with HAART. *J Int Assoc Physicians AIDS Care (Chic)*; 1: 20-25.

Mercier S, Gueye NF, Cournil A, *et al*. (2009). Lipodystrophy and metabolic disorders in HIV-1-infected adults on 4- to 9-year antiretroviral therapy in Senegal: a case-control study. *J Acquir Immune Defic Syndr*; 51: 224-230.

Messner B, Bernhard D (2014). Smoking and cardiovascular disease: mechanisms of endothelial dysfunction and early atherogenesis. *Arterioscler Thromb Vasc Biol*; 34: 509-515.

Millán J, Pintó X, Muñoz A, *et al*. (2009). Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. *Vasc Health Risk Manag;* 5: 757-765.

Mills EJ, Bakanda C, Birungi J, *et al*. (2011). Life expectancy of persons receiving combination antiretroviral therapy in low-income countries: a cohort analysis from Uganda. *Ann Intern Med*; 155: 209-216.

Mingote E, Urrutia A, Viteri A, *et al*. (2013). Graves' disease as a late manifestation of immune reconstitution syndrome after highly active antiretroviral therapy in an HIV-1

infected patient. *World J AIDS*; 3: 187-191.

Ministry of Medical Services, Republic of Kenya. Guidelines for antiretroviral therapy in Kenya. (URL http://healthservices./healthservices/Kenya%20Treatment%20Guidelines.pdf). Accessed 23 April 2015.

Mirtz TA, Morgan L, Wyatt LH, Leon Greene L (2009). An epidemiological examination of the subluxation construct using Hill's criteria of causation. *Chiropr Osteopat*; 17: 13.

Mital P, Goyal LK, Saini HL, *et al*. (2013). Metabolic syndrome and sub clinical atherosclerosis: influence of HIV status and HAART. *Sch J App Med Sci*; 1: 830-836.

Mittal A, Achappa B, Madi D, *et al*. (2013). The development of metabolic risk factors after the initiation of the second line anti- retroviral therapy. *J Clin Diagn Res*; 7: 265-268.

Mohammed AE, Shenkute TY, Gebisa WC (2015). Diabetes mellitus and risk factors in human immunodeficiency virus-infected individuals at Jimma University Specialized Hospital, Southwest Ethiopia. *Diabetes Metab Syndr Obes*; 8: 197-206.

Molina JM, Andrade-Villanueva J, Echevarria J, *et al*. (2010). Once-daily atazanavir/ritonavir compared with twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1-infected patients: 96-week efficacy and safety results of the CASTLE study. *J Acquir Immune Defic Syndr*; 53: 323-332.

Montes ML, Pulido F, Barros C, *et al*. (2005). Lipid disorders in antiretroviral-naïve patients treated with lopinavir/ritonavir-based HAART: frequency, characterization and risk factors. *J Antimicrob Chemoth*; 5: 800-804.

Moutzouri E, Elisaf M, Liberopoulos EN (2011). Hypocholesterolaemia. *Curr Vasc*

Pharmacol; 9: 200-212.

Muhammad S, Sani MU, Okeahialam BN (2013). Cardiovascular disease risk factors among HIV-infected Nigerians receiving highly active antiretroviral therapy. *Niger Med J*; 54:185-190.

Muhammad S, Sani MU, Okeahialam BN (2013). Prevalence of dyslipidaemia among human immunodeficiency virus infected Nigerians. *Ann Afr Med*; 12: 24-28.

Mujawar Z, Rose H, Morrow MP, *et al.* (2006). Human immunodeficiency virus impairs reverse cholesterol transport from macrophages. *PLoS Biol*; 4: e365.

Mulligan K, Grunfeld C, Tai VW, *et al*. (2000). Hyperlipidaemia and insulin resistance are induced by protease inhibitors independent of changes in body composition in patients with HIV infection. *J Acquir Immune Defic Syndr*; 23: 35-43.

Mulrow CD (1994). Systematic Reviews: Rationale for systematic reviews. *BMJ*; 309: 597.

Mustapha KB, Ehianeta TS, Kirim RA, *et al*. (2011). Highly active antiretroviral therapy (HAART) and body mass index (BMI) relationship in people living with HIV/AIDS (PLWHA) in the Federal Capital Territory, Nigeria and the neighbouring states. *J AIDS HIV Res*; 3: 57-62.

Mutimura E, Stewart A, Rheeder P, Crowther NJ (2008). Metabolic function and the prevalence of lipodystrophy in a population of HIV-infected African subjects receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr*; 46: 451-455.

National Agency for the Control of AIDS (2012). Global AIDS response country progress report Nigeria 2012. (URL http://www.aidsmap.com...Global_AIDS_Response_Country_Progress_Report_Nigeria...2012.pdf). Accessed 3 January 2014.

National Agency for the Control of AIDS (2014). HIV prevalence by states. (URL http://naca.gov.ng/new/content/hiv-prevalence-rate-states). Accessed 12 Aug 2015.

National Bureau of Statistics (2012). Vital statistics and cause-specific death rate in nine states and the federal capital territory. (URL http://www.scribd.com/doc/216084784/Demographic-Statistics-Bulletin-2012). Accessed 3 January 2014.

National Institute for Health and Care Excellence (2011). Hypertension: Clinical management of primary hypertension in adults. (URL https://www.nice.org.uk/guidance/cg127/chapter/guidance). Accessed 12 Aug 2015.

National Institute for Health and Clinical Excellence (2011). Public health draft guidance: assessing body mass index and waist circumference thresholds for intervening to prevent ill health and premature death among adults from black, Asian and other minority ethnic groups in the UK. (URL https://www.nice.org.uk/guidance/ph46/documents/bmi-and-waist-circumference-black-and-minority-ethnic-groups-draft-guidance2). Accessed 04 November 2015.

Nduka C, Sarki A, Uthman O, *et al*. (2015). Impact of antiretroviral therapy on serum lipoprotein levels and dyslipidaemias: A systematic review and meta-analysis. *Int J Cardiol*; 199: 307-318.

Nduka CU, Uthman OA, Kimani PK, Stranges S (2015). Drug abuse in people living with HIV in the era of highly active antiretroviral therapy: a systematic review and meta-analysis. *J Addict Res Ther*; 6: 255.

Nduka CU, Stranges S, Sarki AM, Kimani PK, Uthman OA (2015). Evidence of increased blood pressure and hypertension risk among people living with HIV on antiretroviral

therapy: a systematic review with meta-analysis. *J Hum Hypertens*; doi:10.1038/jhh.2015.97.

Ngala RA, Fianko K (2013). Effects of HIV infection and antiretroviral therapy on cardiovascular risk factors. *Trends in Mol Sci*; 10: 1-7.

Ngatchou W, Lemogoum D, Ndobo P, *et al*. (2013). Effects of antiretroviral therapy on arterial stiffness in Cameroonian HIV-infected patients. *Blood Press Monit*; 18: 247-251.

Ngondi J, Mbouobda H, Fonkoua M, *et al*. (2007). The Long-term Effect of Different Combination Therapies on Glucose Metabolism in HIV/Aids Subjects in Cameroon. *J Med Sci*; 7: 609-614.

Nguyen KA, Peer N, Mills EJ, Kenge PA (2016). A meta-analysis of metabolic syndrome prevalence in the global HIV-infected population. *PLoS ONE*; 11: e0150970.

Nuesch R, Wang Q, Elzi L, *et al*. (2013). Risk of cardiovascular events and blood pressure control in hypertensive HIV-infected patients: Swiss HIV Cohort Study (SHCS). *J Acquir Immune Defic Syndr;* 62*:* 396-404.

Nzou C, Kambarami R, Onyango F, *et al*. (2010). Clinical predictors of low CD4 count among HIV infected pulmonary tuberculosis clients: a health facility-based survey. *S Afr Med J*; 100: 602-605.

Ogundahunsi OA, Oyegunle VA, Ogun SA, *et al*. (2008). HAART and lipid metabolism in a resource poor West African setting. *Afr J Biomed Res*; 11: 27-31.

Ogunmola OJ, Oladosu OY, Olamoyegun AM (2014). Association of hypertension and obesity with HIV and antiretroviral therapy in a rural tertiary health centre in Nigeria: a cross-sectional cohort study. *Vasc Health Risk Manag*; 10: 129-137.

Orlando G, Meraviglia P, L Cordier L, *et al*. (2006). Antiretroviral treatment and age-related

comorbidities in a cohort of older HIV-infected patients. *HIV Med*; 7: 549-557.

Osborne J (2002). Notes on the use of data transformations. *Pract Asses Res Eval*; 8.

Oshinaike O, Akinbami A, Ojelabi O, *et al*. (2014). Quality of sleep in an HIV population on antiretroviral therapy at an urban tertiary centre in Lagos, Nigeria. *Neurology Research International*; 2014: 298703.

Owiredu WK, Quaye L, Amidu N (2011). Oxidative stress and dyslipidaemia among Ghanaian HAART-naïve HIV patients and those on HAART. *West Afr J Pharm*; 22:58-66.

Pace-Schott EF, Spencer RM (2011). Age-related changes in the cognitive function of sleep. *Prog Brain Res*; 191: 75-89.

Palacios R, Santos J, Garcı´a A, *et al*. (2006). Impact of highly active antiretroviral therapy on blood pressure in HIV-infected patients. A prospective study in a cohort of naive patients. *HIV Med*; 7: 10-15.

Peck RN, Shedafa R, Kalluvya S, *et al*. (2014). Hypertension, kidney disease, HIV and antiretroviral therapy among Tanzanian adults: a cross-sectional study. *BMC Med*; 12: 1-11.

Pefura Yone EW, Betyoumin AF, Kengne AP, *et al*. (2011). First line antiretroviral therapy and dyslipidaemia in people living with HIV-1 in Cameroon: a cross-sectional study. *AIDS Res Ther*; 8: 1-8.

Pence BW, Miller WC, Whetten K, *et al*. (2006). Prevalence of DSM-IV-defined mood, anxiety, and substance use disorders in an HIV clinic in the South-Eastern United States. *J Acquir Immune Defic Syndr*; 42: 298-306.

Pereira TV, Horwitz RI, Loannidis JP (2012). Empirical evaluation of very large treatment effects of medical interventions. *JAMA*; 308: 1676-1684.

Petoumenos K, Reiss P, Ryom, L, *et al*. (2014). Increased risk of cardiovascular disease

(CVD) with age in HIV-positive men: a comparison of the D:A:D CVD risk equation and general population CVD risk equations. *HIV Med*; DOI:10.1111/hiv.12162.

Pittilo RM (2000). Cigarette smoking, endothelial injury and cardiovascular disease. *Int J Exp Pathol*; 81: 219-230.

PrayGod G, Range N, Faurholt-Jepsen D, *et al*. (2011). Weight, body composition and handgrip strength among pulmonary tuberculosis patients: a matched cross-sectional study in Mwanza, Tanzania. *Trans R Soc Trop Med Hyg*, 105: 140-147.

Preacher KJ, Hayes AF (2008). Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behav Res Methods*; 40: 879-891.

Pujari SN, Dravid A, Naik E, *et al*. (2005). Lipodystrophy and dyslipidemia among patients taking first-line, World Health Organization-recommended highly active antiretroviral therapy regimens in Western India. *J Acquir Immune Defic Syndr*; 39: 199.

Puranik R, Celermajer DS (2003).Smoking and endothelial function. *Prog Cardiovasc Dis*; 45: 443-458.

Radloff LS (1977). The CES-D Scale: A self-report depression scale for research in the general population. *Appl Psychol Meas*; 1: 385-401.

Rasmussen TA, Tolstrup M, Melchjorsen J, *et al*. (2011). Evaluation of cardiovascular biomarkers in HIV-infected patients switching to abacavir or tenofovir based therapy. *BMC Infect Dis*; 11: 267.

Rathbun RC, Liedtke MD, Lockhart SM (2013). Antiretroviral therapy for HIV infection. (URL http://emedicine.medscape.com/article/1533218-overview). Accessed 20 May 2015.

Riddler SA, Li X, Chu H, *et al*. (2007). Longitudinal changes in serum lipids among HIV-infected men on highly active antiretroviral therapy. *HIV Medicine*; 8: 280-287.

Riddler SA, Smit E, Cole SR, *et al*. (2003). Impact of HIV infection and HAART on serum lipids in Men. *JAMA*; 289: 2978-2982.

Robins JM, Greenland S (1992). Identifiability and exchangeability for direct and indirect effects. *Epidemiology*; 3: 143-155.

Rokx C, Verbon A, Rijnders BJ (2015). Short communication: Lipids and cardiovascular risk after switching HIV-1 patients on nevirapine and emtricitabine/tenofovir-DF to rilpivirine/emtricitabine/tenofovir-DF. *AIDS Res Hum Retroviruses*; 31: 363-367.

Rosenbaum PR (1987). The role of a second control group in an observational study. *Statistical Science*; 2: 292-316.

Rosenbaum PR, Rubin DB (1985). Constructing a control group using multivariate matched sampling methods that incorporate the propensity score. *The American Statistician*; 39: 33-38.

Rothman KJ (2002). Epidemiology: An Introduction. Oxford University Press: New York.

Rothstein HR, Sutton AJ, Borenstein M (2005). Publication Bias in Meta-Analysis – Prevention, Assessment and Adjustments: John Wiley & Sons: London.

Rubin DB (1974). Estimating causal effects of treatment in randomised and non-randomised studies. *J Educat Psychol*; 66: 688-701.

Rubin DB, Thomas N (2000). Combining propensity score matching with additional adjustments for prognostic covariates. *J Am Stat Assoc*; 95: 573-585.

Rucker DD, Preacher KJ, Tormala ZL, Petty RE (2011). Mediation Analysis in Social Psychology: Current Practices and New Recommendations. *Social and Personality Psychology Compass*; 5: 359-371.

Sabin CA (2013). Do people with HIV infection have a normal life expectancy in the era of combination antiretroviral therapy? *BMC Med*; 11: 251.

Samaras K, Wand H, Matthew L, *et al*. (2007). Prevalence of metabolic syndrome in HIV-infected patients receiving highly active antiretroviral therapy using International Diabetes Foundation and Adult Treatment Panel III criteria: Associations with insulin resistance, disturbed body fat compartmentalization, elevated C-reactive protein, and hypoadiponectinemia. *Diabetes care*; 30: 113-119.

Samet JH, Horton NJ, Meli S, *et al*. (2004). Alcohol consumption and antiretroviral adherence among HIV-infected persons with alcohol problems. *Alcohol Clin Exp Res*; 28: 572-577.

Sarki A, Nduka C, Stranges S, *et al*. (2015). Prevalence of hypertension in low- and middle-income countries: a systematic review and meta-analysis. *Medicine*; 94: e1959.

Savès M, Chêne G, Ducimetière P, *et al*. (2003). Risk Factors for Coronary Heart Disease in Patients Treated for Human Immunodeficiency Virus Infection Compared with the General Population. *Clin Infect Dis*; 37: 292-298.

Scarcella P, Buonomo E, Zimba I, *et al*. (2011). The impact of integrating food supplementation, nutritional education and HAART (Highly Active Antriretroviral Therapy) on the nutritional status of patients living with HIV/AIDS in Mozambique: results from the DREAM Programme. *Ig Sanita Pubbl*; 67: 41-52.

Schneeweiss S (2010). A basic study design for expedited signal evaluation based on electronic healthcare data. *Pharmacoepidemiol Drug Saf*; 19: 858-868.

Schoenbach VJ (2000). Multicausality: effect modification. (URL http://www.epidemiolog.net/evolving/Multicausality-EffectModification.pdf). Accessed 20

February 2016.

Scholten F, Mugisha J, Seeley J, *et al*. (2011). Health and functional status among older people with HIV/AIDS in Uganda. *BMC Public Health*; 11: 1-10.

Seaberg EC, Muñoz A, Lu M, *et al*. (2005). Association between highly active antiretroviral therapy and hypertension in a large cohort of men followed from 1984 to 2003. *AIDS*; 19: 953-960.

Seeger JD, Kurth T, Walker AM (2007). Use of propensity score technique to account for exposure-related covariates: an example and lesson. *Medical Care*; 45: S143–S148.

Selvin E, Steffes MW, Zhu H, *et al*. (2010). Glycated Hemoglobin, Diabetes, and Cardiovascular Risk in Nondiabetic Adults. *N Engl J Med*; 362: 800-811.

Shahmanesh M, Das S, Stolinski M, *et al*. (2005). Antiretroviral treatment reduces very-low-density lipoprotein and intermediate-density lipoprotein apolipoprotein b fractional catabolic rate in human immunodeficiency virus-infected patients with mild dyslipidemia. *J Clin Endocrinol Metab*; 90: 755-760.

Shapiro RL, Souda S, Parekh N, *et al*. (2012). High prevalence of hypertension and placental insufficiency, but no in utero HIV transmission, among women on HAART with stillbirths in Botswana. *PLoS One*; 7: 1-7.

Shen Y, Wang Z, Liu Li, *et al*. (2013). Prevalence of hyperglycaemia among adults with newly diagnosed HIV/AIDS in China. *BMC Infect Dis*; 13: 79.

Shlay JC, Bartsch G, Peng G, *et al.* (2007). Long-term body composition and metabolic changes in antiretroviral naive persons randomized to protease inhibitor-, nonnucleoside reverse transcriptase inhibitor-, or protease inhibitor plus nonnucleoside reverse transcriptase inhibitor-based strategy. *J Acquir Immune Defic Syndr*; 44: 506-517.

Shlisky JD, Hartman TJ, Kris-Etherton PM, *et al*. (2012). Partial sleep deprivation and energy balance in adults: an emerging issue for consideration by dietetics practitioners. *J Acad Nutr Diet*; 112: 1785-1797.

Shor-Posner G, Basit A, Lu Y, *et al*. (1993). Hypocholesterolaemia is associated with immune dysfunction in early human immunodeficiency virus-1 infection. *Am J Med*; 94: 515.

Shrier I, Boivin J, Steele RJ, *et al.* (2007). Should meta-analyses of interventions include observational studies in addition to randomized controlled trials? a critical examination of underlying principles. *Am J Epidemiol*; 166: 1203-1209.

Shuter J, Bernstein S (2008). Cigarette smoking is an independent predictor of nonadherence in HIV infected individuals receiving highly active antiretroviral therapy. *Nicotine Tob Res*; 10: 731-736.

Silva EF, Bassichetto KC, Lewi DS (2009). Lipid profile, cardiovascular risk factors and metabolic syndrome in a group of AIDS patients. *Arquivos Brasileiros de Cardiologia*; 93: 113-118.

Silva EF, Lewi DS, Vedovato GM, *et al*. (2010). Nutritional and clinical status, and dietary patterns of people living with HIV/AIDS in ambulatory care in Sao Paulo, Brazil. *Rev Bras Epidemiol*; 13: 677-688.

Singh J, Verma M, Ghalaut PS, *et al*. (2014). Alteration in lipid profile in treatment-naïve HIV-infected patients and changes following HAART initiation in Haryana. *J Endocrinol. Metab*; 4: 25-31.

Sivo SA, Xitao Fan E, Witta L, *et al*. (2006). The search for "optimal" cut-off properties: fit index criteria in structural equation modelling. *J Exp Educ*; 74: 267-288.

Smith CJ, Levy I, Sabin CA, *et al* (2004). Cardiovascular disease risk factors and antiretroviral therapy in an HIV-positive UK population. *HIV Med*; 5: 88-92.

Soares LR, da Silva DC, Gonsalez CR, *et al*. (2015). Discordance between body mass index and anthropometric measurements among HIV-1-infected patients on antiretroviral therapy and with lipoatrophy/lipohypertrophy syndrome. *Rev Inst Med Trop Sao Paulo*; 57: 105-110.

Søgaard OS, Schønheyder HC, Bukh AR, *et al*. (2010). Pneumococcal conjugate vaccination in persons with HIV: the effect of highly active antiretroviral therapy. *AIDS*; 24: 1315-1322.

SoRelle R (1998). Vascular and lipid syndromes in selected HIV-infected patients. *Circulation*; 98: 829-830.

Sreekantamurthy GG, Singh NB, Singh TB, *et al*. (2014). Study of body composition and metabolic parameters in HIV-1 male patients. *J Nutr Metab*; (Suppl. 498197): 1-5.

Stamler J, Stamler R, Neaton JD (1993). Blood pressure, systolic and diastolic, and cardiovascular risks: US population data. *Arch Intern Med*; 153: 598-615.

Stamler R (1991). Implications of the INTERSALT study. *Hypertension*; 17: 16-20.

Stanley TL, Grinspoon SK (2012). Body Composition and Metabolic Changes in HIV-Infected Patients. *J Infect Dis*; 205: S383-S390.

Steichen TJ, Egger M, Sterne JAC (1998). Tests for publication bias in meta-analysis. *The Stata Technical Bulletin*; STB-44: 3-4.

Stein JH (2003). Dyslipidemia in the era of HIV protease inhibitors. *Prog Cardiovasc Dis*; 45:293-304.

Sterne JAC, Harbord RM (2004). Funnel plots in meta-analysis. *The State Journal*; 4: 127-141.

Sterne JAC, Higgins JP, Reves BC (2014). A Cochrane risk of bias assessment tool: for non-randomized studies of interventions. (URL http://www.riskofbias.info). Accessed 06 November 2015.

Sterne JAC, Sutton AJ, Loannidis JPA, *et al*. (2011). Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ*; 343: d4002.

Stewart AL, Ware JE (1992). Measuring functioning and wellbeing: the Medical Outcomes Study approach. Duke University Press: Durham, NC

Steyerberg EW (2009). Clinical prediction models: a practical approach to development, validation and updating. Springer: New York.

Stranges S, Dorn JM, Shipley MJ, *et al*. (2008). Correlates of short and long sleep duration: a cross-cultural comparison between the United Kingdom and the United States. *Am J Epidemiol*; 168: 1353-1364.

Stranges S, Donahue RP (2015). Health-related quality of life and risk of hypertension in the community: prospective results from the Western New York Health Study. *J Hypertens*; 33: 720-726.

Strategies for Management of Antiretroviral Therapy Study Group (2006). CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med*; 355: 2283-2296.

Tadewos A, Addis Z, Ambachew H, Banerjee S (2012). Prevalence of dyslipidaemia among HIV-infected patients using first-line highly active antiretroviral therapy in Southern Ethiopia: a cross-sectional comparative group study. *AIDS Res Ther*; 9: 1-8.

Takarabe D, Rokukawa Y, Takahashi Y, *et al*. (2010). Autoimmune diabetes in HIV-infected patients on highly active antiretroviral therapy. *Journal of Clin Endocrinol Metab*; 95: 4056-4060.

Tamang HK, Timilsina U, Singh KP, *et al*. (2014). Apo B/Apo A-I Ratio is statistically a better predictor of cardiovascular disease (CVD) than conventional lipid profile: A study from Kathmandu Valley, Nepal. *J Clin Diagn Res*; 8: 34-36.

Tapp C, Milloy MJ, Kerr T, *et al*. (2011). Female gender predicts lower access and adherence to antiretroviral therapy in a setting of free healthcare. *BMC Infect Dis*; 11: 86.

Tassie JM, Malateste K, Pujades-Rodríguez M, *et al*. (2010). Evaluation of three sampling methods to monitor outcomes of antiretroviral treatment programmes in low- and middle-income countries. *PLoS One*; 5: e13899.

Tate T, Willig AL, Willig JH, *et al*. (2012). HIV infection and obesity: Where did all the wasting go? *Antivir Ther*; 17: 1281-1289.

Tesfaye DY, Kinde S, Medhin G, *et al*. (2014). Burden of metabolic syndrome among HIV-infected patients in Southern Ethiopia. *Diabetes Metab Syndr*; 8: 102-107.

The World Bank Group (2016). Country and lending groups. In: Data. World Bank. (URL http://data.worldbank.org/about/country-and-lending-groups). Accessed 29 January 2016.

Thenappan T, Roy SS, Duval S, *et al*. (2014). Beta-Blocker therapy is not associated with adverse outcomes in patients with pulmonary arterial hypertension: a propensity score analysis. *Circulation: Heart Failure*; doi:10.1161/CIRCHEARTFAILURE.114.001429.

Thiebaut R, El-Sadr WM, Friis-Moller N, *et al*. (2003). Predictors of hypertension and changes in blood pressure in HIV-infected patients. *Antivir Ther*; 10: 811-823.

Thompson SG, Higgins JP (2002). How should meta-regression analyses be undertaken and

interpreted? *Stat Med*; 21: 1559-1573.

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

Troll JG (2011). Approach to dyslipidaemia, lipidodystrophy, and cardiovascular risk in patients with HIV infection. *Curr Atheroscler Rep*; 13: 51-56.

University of California Los Angeles Institute for Digital Research and Education (2016). What are pseudo R-squareds? (URL http://www.ats.ucla.edu/stat/mult_pkg/faq/general/Psuedo_RSquareds.htm). Accessed 20 February 2016.

University of California Los Angeles Institute for Digital Research and Education (2016). What statistical analysis should I use? Statistical analyses using Stata. (URL http://www.ats.ucla.edu/stat/stata/whatstat/whatstat.htm). Accessed 20 February 2016.

University of California San Francisco Centre for HIV Information (2014). Database for antiretroviral drug reactions. Interactions between cardiovascular medications and antiretrovirals. (URL http://hivinsite.ucsf.edu/insite?page=ar-00-02&post=10&param=18). Accessed 2 February 2016.

University of California San Francisco Centre for HIV Information (2016). Adherence to antiretroviral therapy. (URL http://hivinsite.ucsf.edu/InSite?page=kb-03-02-09). Accessed 24 March 2016.

Utulu SN, Lawoyin TO (2007). Epidemiological features of HIV infection among pregnant women in Makurdi, Benue State Nigeria. *J Biosoc Sci*; 39: 397-408.

Van Leth F, Phanuphak P, Stoes E, *et al*. (2004). Nevirapine and Efavirenz elicit different

changes in lipid profiles in antiretroviral-therapy-naive patients infected with HIV-1. *PLOS Med* 1: e19.

Van Pelt RE, Evans EM, Schechtman KB, *et al*. (2001). Waist circumference vs body mass index for prediction of disease risk in postmenopausal women. *Int J Obes Relat Metab Disord*; 25: 1183-1188.

Velentgas P, Dreyer NA, Nourjah P, *et al*. (2013). Developing a Protocol for Observational Comparative Effectiveness Research: A User's Guide. Agency for Healthcare Research and Quality: Rockville, Massachusetts.

Wagenseil JE, Mecham RP (2012). Elastin in large artery stiffness and hypertension. J Cardiovasc Transl Res; 5: 264-273.

Wanke C, Gerrior J, Hendricks K, *et al*. (2005). Alterations in lipid profiles in HIV-infected patients treated with protease inhibitor therapy are not influenced by diet. *Nutr Clin Pract*; 20: 66-68.

Ware JE, Kosinski M, Keller SD (1996). A 12-Item Short-Form Health Survey: Construction of scales and preliminary tests of reliability and validity. *Medical Care;* 34:220-233.

Wassertheil-Smoller S, Shumaker S, Ockene J, *et al*. (2004). Depression and cardiovascular sequelae in postmenopausal women. The Women's Health Initiative (WHI). *Arch Intern Med*; 164: 289-298.

Weerakkody MI, Buddhakorala K, Somasundaram NP (2013). Diabetes and metabolic complications among patients with HIV and AIDS. *Sri Lanka J Diabetes Endocrinol Metab*; 3: 68-75.

Welch BL (1951). On the comparison of several mean values: An alternative approach. *Biometrika*; 38: 330–336.

Wester CW, Koethe JR, Shepherd BE, *et al*. (2011). Non-AIDS-defining events among HIV-1-infected adults receiving combination antiretroviral therapy in resource-replete versus resource-limited urban settings. *AIDS*; 23: 1471-1479.

Wibbeler T, Reichelt D, Husstedt IW, Evers S (2012). Sleepiness and sleep quality in patients with HIV infection. *J Psychosom Res*; 72: 439-442.

Wilkinson R, Marmot M (2003). Social determinants of health: the solid facts. WHO Press: Copenhagen, Denmark.

Wilson SL, Scullard G, Fidler SJ, *et al*. (2009). Effects of HIV status and antiretroviral therapy on blood pressure. *HIV Med*; 10: 388-394.

Woerle HJ, Mariuz PR, Meyer C, *et al*. (2003). Mechanisms for the deterioration in glucose tolerance associated with HIV protease inhibitor regimens. *Diabetes*; 52: 918-925.

Womack J, Tien PC, Feldman J, *et al*. (2007). Obesity and Immune Cell Counts in Women. *Metabolism*; 56: 998-1004.

World Health Organisation & International Diabetes Federation (2006). Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. (URL https://www.idf.org/webdata/docs/WHO_IDF_definition_diagnosis_of_diabetes.pdf). Accessed 16 August 2015.

World Health Organisation (2010). Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: recommendation for a public health approach. (URL http://apps.who.int/iris/bitstream/10665/75236/1/9789241599818_eng.pdf). Accessed 31 March 2016.

World Health Organisation (2016). Consolidated ARV guidelines 2013. (URL http://www.who.int/hiv/pub/guidelines/arv2013/art/statartadolescents_rationale/en/).

Accessed 7 April 2015.

World Health Organization (1946). Preamble to the constitution of the World Health Organization. Vol. 2, p. 100. International Health Conference; New York.

World Health Organization (1999). Guidelines set new definitions, update treatment for hypertension. (URL http://www.who.int/bulletin/archives/77(3)293.pdf). Accessed 28 November 2014.

World Health Organization (2001). WHO STEPS Instrument (Core and Expanded). The WHO STEPwise approach to chronic disease risk factor surveillance. (URL http://www.who.int/chp/steps/STEPS_Instrument_v2.1.pdf). Accessed 20 September 2015.

World Health Organization (2005). Treat 3 million by 2005: interim WHO clinical staging of HIV/AIDS and HIV/AIDS case definitions for surveillance. (URL http://www.who.int/hiv/pub/guidelines/clinicalstaging.pdf). Accessed 14 January 2016.

World Health Organization (2010). Global recommendations on physical activity for health. (URL http://whqlibdoc.who.int/publications/2010/9789241599979_eng.pdf?ua=1). Accessed 02 July 2015.

World Health Organization (2013). Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. (URL http://www.who.int/hiv/mediacentre/feature_story/hiv_arv2013/en/). Accessed 7 April 2015.

World Health Organization (2015a). Antiretroviral therapy (ART) coverage among all age groups. (URL http://www.who.int/gho/hiv/epidemic_response/ART_text/en/). Accessed 4 November 2015.

World Health Organization (2015b). Top ten causes of death. (URL

http://www.who.int/mediacentre/factsheets/fs310/en/). Accessed 30 March 2016).

World Heart Federation (2015a). Cardiovascular disease risk factors. (URL http://www.world-heart-federation.org/cardiovascular-health/cardiovascular-disease-risk-factors/). Accessed 30 March 2016.

World Heart Federation (2015b). Hypertension. (URL http://www.world-heart-federation.org/cardiovascular-health/cardiovascular-disease-risk-factors/hypertension/). Accessed 9 December 2015.

Worm SW, Sabin CA, Reiss P, *et al*. (2009). Presence of the Metabolic Syndrome Is Not a Better Predictor of Cardiovascular Disease Than the Sum of Its Components in HIV-Infected Individuals. *Diabetes Care*; 32: 474-480.

Wright S (1921). Correlation and causation. Journal of Agricultural Research; 20: 557–585.

Wright S (1934). The method of path coefficients. *Annals of Mathematical Statistics*; 5: pp. 161-215.

Yarasheski KE, Tebas P, Sigmund C, *et al*. (1999). Insulin resistance in HIV protease inhibitor-associated diabetes. *J Acquir Immune Defic Syndr*; 21: 209-216.

Yusuf PS, Hawken S, Ounpuu S, *et al*. (2004). Effect of modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): Case-control study. *Lancet*; 364: 937-952.

Zannou DM, Denoeud L, Lacombe K, *et al*. (2009). Incidence of lipodystrophy and metabolic disorders in patients starting non-nucleoside reverse transcriptase inhibitors in Benin. *Antivir Ther*; 14: 371-380.

Zeng Y, Ye YC, Luo L, *et al*. (2010). Premature atherosclerosis in patients with acquired immunodeficiency syndrome. *Chin Med J (Engl)*; 123: 3396-3399.

Zuo H, Shi Z, Yuan B, *et al*. (2012). Interaction between physical activity and sleep duration in relation to insulin resistance among non-diabetic Chinese adults. *BMC Public Health*; 12: 247.

APPENDICES

APPENDIX 1: Data extraction form

GENERAL INFORMATION	
Researcher performing data extraction	
Date of data extraction	
Author	
Article title	
Citation	
Country of origin	
STUDY CHARACTERISTICS	
Study aims/objectives	
Study design	
Sampling strategy	
Inclusion criteria	
Exclusion criteria	
Exposure	Highly Active Antiretroviral Therapy (HAART)
Comparator	HAART-naïve
PARTICIPANT CHARACTERISTICS	
Mean age	
• *HAART-exposed*	
• *HAART-naïve*	
Gender distribution	
• *HAART-exposed*	

• *HAART-naïve*	
Number of participants	
• *HAART-exposed*	
• *HAART-naïve*	
HAART regimen (%)	
• *NNRTI-based*	
• *PI-based*	
OUTCOME DATA/RESULTS	
Outcomes	
Outcome measures	
Main results (with pooled effect sizes)	
Confounders	

APPENDIX 2

WHO STEPS Instrument

(Core and Expanded)

World Health Organization

The WHO STEPwise approach to chronic disease risk factor surveillance (STEPS)

World Health Organization

20 Avenue Appia, 1211 Geneva 27, Switzerland

STEPS Instrument

Overview

Introduction

This is the generic STEPS Instrument which sites/countries will use to develop their tailored instrument. It contains the:

- CORE items (unshaded boxes)
- EXPANDED items (shaded boxes).

Core Items

The Core items for each section ask questions required to calculate basic variables. For example:

- current daily smokers
- mean BMI.

Note: All the core questions should be asked, removing core questions will impact the analysis.

Expanded items

The Expanded items for each section ask more detailed information. Examples include:

- use of smokeless tobacco
- sedentary behaviour.

Guide to the columns

The table below is a brief guide to each of the columns in the Instrument.

Column	Description	Site Tailoring
Number	This question reference number is designed to help interviewers find their place if interrupted.	Renumber the instrument sequentially once the content has been finalized.
Question	Each question is to be read to the participants	• Select sections to use. • Add expanded and optional questions as desired.
Response	This column lists the available response options which the interviewer will be circling or filling in the text boxes. The skip instructions are shown on the right hand side of the responses and should be carefully followed during interviews.	• Add site specific responses for demographic responses (e.g. C6). • Change skip question identifiers from code to question number.
Code	The column is designed to match data from the instrument into the data entry tool, data analysis syntax, data book, and fact sheet.	This should never be changed or removed. The code is used as a general identifier for the data entry and analysis.

Participant Identification Numb ⌊_⌊_⌋ ⌊_⌊_⌋ ⌊_⌊_⌋

WHO STEPS Instrument

for Chronic Disease Risk Factor Surveillance

<insert country/site name>

Survey Information

Location and Date		Response	Code
1	Cluster/Centre/Village ID	⌊_⌊_⌋	I1
2	Cluster/Centre/Village name		I2
3	Interviewer ID	⌊_⌊_⌋	I3
4	Date of completion of the instrument	⌊_⌊_⌋ ⌊_⌊_⌋ ⌊_⌊_⌊_⌊_⌋ dd mm year	I4

Participant Id Number

Consent, Interview Language and Name		Response	Code
5	Consent has been read and obtained	Yes 1 No 2 **If NO, END**	I5
6	Interview Language *[Insert Language]*	English 1 *[Add others]* 2 *[Add others]* 3 *[Add others]* 4	I6
7	Time of interview (24	⌊_⌊_⌋ : ⌊_⌊_⌋ hrs mins	I7
8	Family Surname		I8
9	First Name		I9
Additional Information that may be helpful			
10	Contact phone number where possible		I10

Record and file identification information (I5 to I10) separately from the completed questionnaire.

Participant Identification Numb ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋

Step 1 Demographic Information

CORE: Demographic Information			
Question		**Response**	**Code**
11	Sex (*Record Male / Female as observed)*	Male 1 Female 2	C1
12	What is your date of birth? *Don't Know 77 77 7777*	⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ *If known, Go to C4* dd mm year	C2
13	How old are you?	Years ⌊_⌋⌊_⌋	C3
14	In total, how many years have you spent at school or in full-time study (excluding	Years ⌊_⌋⌊_⌋	C4

EXPANDED: Demographic Information			
15	What is the **highest level of education** you have completed? *[INSERT COUNTRY-SPECIFIC CATEGORIES]*	No formal schooling 1 Less than primary school 2 Primary school completed 3 Secondary school completed 4 High school completed 5	C5
16	What is your *[insert relevant ethnic group / racial group / cultural subgroup / others]* **background**?	[*Locally defined]* 1 [*Locally defined]* 2 [*Locally defined]* 3 Refused 88	C6
17	What is your **marital status**?	Never married 1 Currently married 2 Separated 3 Divorced 4 Widowed 5 Cohabitating 6 Refused 88	C7
18	Which of the following best describes your **main work** status over the past 12 months? *[INSERT COUNTRY-SPECIFIC CATEGORIES]* *(USE SHOWCARD)*	Government employee 1 Non-government employee 2 Self-employed 3 Non-paid 4 Student 5 Homemaker 6 Retired 7 Unemployed (able to Unemployed (unable to work) 9 Refused 88	C8
19	How many people older than 18 years, including yourself, live in your	Number of people ⌊_⌋⌊_⌋	C9

EXPANDED: Demographic Information, Continued			
Question		**Response**	**Code**
20	Taking **the past year**, can you tell me what the average earnings of the household have been? *(RECORD ONLY ONE, NOT ALL 3)*	Per week ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋ *Go to T1*	C10a
		OR per month ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋ *Go to T1*	C10b
		OR per year ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋ *Go to T1*	C10c
		Refused 88	C10d
21	If you don't know the amount, can you give an **estimate** of the annual household income if I read some options to you? Is it *[INSERT QUINTILE VALUES IN LOCAL CURRENCY] (READ OPTIONS)*	≤ Quintile (Q) 1 1 More than Q 1, ≤ Q 2 2 More than Q 2, ≤ Q 3 3 More than Q 3, ≤ Q 4 4 More than Q 4 5 Don't Know 77 Refused 88	C11

Step 1 Behavioural Measurements

CORE: Tobacco Use			
Now I am going to ask you some questions about various health behaviours. This includes things like smoking, drinking alcohol, eating fruits and vegetables and physical activity. Let's start with tobacco.			
Question		**Response**	**Code**
22	Do you currently smoke any **tobacco products**, such as cigarettes, cigars or pipes? *(USE SHOWCARD)*	Yes 1 No 2 *If No, go to T6*	T1
23	Do you currently smoke tobacco products **daily**?	Yes 1 No 2 *If No, go to T6*	T2
24	How old were you when you **first started** smoking daily?	Age (years) Don't know 77 ⌊_⌋⌊_⌋ *If Known,*	T3
25	Do you remember how long ago it was? *(RECORD ONLY 1, NOT ALL 3)* *Don't know 77*	In Years ⌊_⌋⌊_⌋ *If Known, go to T5a*	T4a
		OR in Months ⌊_⌋⌊_⌋ *If Known, go to T5a*	T4b
		OR in Weeks ⌊_⌋⌊_⌋	T4c
26	On average, **how many** of the following do you smoke each day? *(RECORD FOR EACH TYPE, USE SHOWCARD)* *Don't Know 77*	Manufactured cigarettes ⌊_⌋⌊_⌋	T5a
		Hand-rolled cigarettes ⌊_⌋⌊_⌋	T5b
		Pipes full of tobacco ⌊_⌋⌊_⌋	T5c
		Cigars, cheroots, cigarillos ⌊_⌋⌊_⌋	T5d
		Other *If Other, go to T5other*	T5e
		Other (please specify): ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋ *Go to T9*	T5other

Participant Identification Numb ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋

EXPANDED: Tobacco Use			
Question		**Response**	**Code**
27	In the past, did you **ever** smoke **daily**?	Yes 1 No 2 *If No, go to T9*	T6
28	How old were you when you **stopped** smoking **daily**?	Age (years) Don't Know 77 ⌊_⌋⌊_⌋ *If Known, go to*	T7
29	How **long ago** did you stop smoking daily? *(RECORD ONLY 1, NOT ALL 3)* *Don't Know 77*	Years ago ⌊_⌋⌊_⌋ *If Known, go to T9*	T8a
		OR Months ago ⌊_⌋⌊_⌋ *If Known, go to T9*	T8b
		OR Weeks ago ⌊_⌋⌊_⌋	T8c
30	Do you **currently use** any **smokeless tobacco** such as *[snuff, chewing tobacco, betel]*? *(USE*	Yes 1 No 2 *If No, go to T12*	T9
31	Do you **currently use smokeless tobacco** products **daily?**	Yes 1 No 2 *If No, go to T12*	T10
32	On average, how many **times a day** do you use …. *(RECORD FOR EACH TYPE, USE SHOWCARD)* *Don't Know 77*	Snuff, by mouth ⌊_⌋⌊_⌋	T11a
		Snuff, by nose ⌊_⌋⌊_⌋	T11b
		Chewing tobacco ⌊_⌋⌊_⌋	T11c
		Betel, quid ⌊_⌋⌊_⌋	T11d
		⌊_⌋⌊_⌋ *If Other, go to T11other, else go to T13*	T11e
		Other (specify) ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋ *Go to T13*	T11other
33	In the **past**, did you **ever use** smokeless tobacco such as *[snuff, chewing tobacco, or betel]* **daily**?	Yes 1 No 2	T12
34	During the past 7 days, on how many days did someone **in your home** smoke when you were present?	Number of days Don't know 77 ⌊_⌋⌊_⌋	T13
35	During the past 7 days, on how many days did someone smoke in closed areas **in your workplace** (in the building, in a work area or a specific	Number of Don't know or don't work in a closed area ⌊_⌋⌊_⌋	T14

Participant Identification Numb ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋

CORE: Alcohol Consumption			
The next questions ask about the consumption of alcohol.			
Question		**Response**	**Code**
36	Have you **ever** consumed an alcoholic drink such as beer, wine, spirits, fermented cider or *[add other local examples]*? *(USE SHOWCARD OR SHOW*	Yes 1 No 2 *If No, go to D1*	A1a
37	Have you consumed an alcoholic drink within the **past 12 months**?	Yes 1 N 2 *If No, go to D1*	A1b
38	During the past 12 months, **how frequently** had at least one alcoholic drink? *(READ RESPONSES, USE SHOWCARD)*	Daily 1 5-6 days per week 2 1-4 days per week 3 1-3 days per month 4 Less than once a month 5	A2
39	Have you consumed an alcoholic drink within the **past 30 days**?	Yes 1 N 2 *If No, go to D1*	A3
40	During the past 30 days, on how many **occasions** did you have at least one alcoholic drink?	Number Don't know 77 ⌊_⌋⌊_⌋	A4
41	During the past 30 days, when you drank alcohol, **on average**, how many **standard alcoholic drinks** did you have during one drinking occasion?	Number Don't know 77 ⌊_⌋⌊_⌋	A5
42	During the past 30 days, what was the **largest number** of standard alcoholic drinks you had on a single occasion, counting all types of alcoholic drinks together?	Largest number Don't Know 77 ⌊_⌋⌊_⌋	A6
43	During the past 30 days, how many times did you have for **men**: **five or more** for **women**: **four or more**	Number of times Don't ⌊_⌋⌊_⌋	A7

EXPANDED: Alcohol Consumption			
44	During the past 30 days, when you consumed an alcoholic drink, how often was it with meals? Please do not count snacks.	Usually with 1 meals Sometimes 2 Rarely with meals 3 Never with meals 4	A8
45	During each of the **past 7 days**, how many standard alcoholic drinks did you have each day? *(USE SHOWCARD)* *Don't Know 77*	Monday ⌊_⌋⌊_⌋	A9a
		Tuesday ⌊_⌋⌊_⌋	A9b
		Wednesday ⌊_⌋⌊_⌋	A9c
		Thursday ⌊_⌋⌊_⌋	A9d
		Friday ⌊_⌋⌊_⌋	A9e
		Saturday ⌊_⌋⌊_⌋	A9f
		Sunday ⌊_⌋⌊_⌋	A9g

CORE: Diet

The next questions ask about the fruits and vegetables that you usually eat. I have a nutrition card here that shows you some examples of local fruits and vegetables. Each picture represents the size of a serving. As you answer these questions please think of a typical week in the last year.

Question		Response	Code
46	In a typical week, on how many days do you **eat fruit**?	Number of days Don't Know 77 ⌊_⌋⌊_⌋ *If Zero days, go to*	D1
47	How many **servings** of fruit do you eat on **one** of those days? (*USE SHOWCARD)*	Number of servings ⌊_⌋⌊_⌋	D2
48	In a typical week, on how many days do you	Number of days Don't Know 77 ⌊_⌋⌊_⌋ *If Zero days, go to*	D3
49	How many **servings** of vegetables do you eat on one of those days? *(USE*	Number of servings ⌊_⌋⌊_⌋	D4

EXPANDED: Diet

50	What type of **oil or fat is most often** used for meal preparation in your household? *(USE SHOWCARD)* *(SELECT ONLY ONE)*	Vegetable oil 1 Lard or suet 2 Butter or ghee 3 Margarine 4 Other 5 *If Other, go to D5 other* None in particular None used 6 Don't know 7	D5
		Other ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋	D5other
51	On average, how many meals per week do you eat that were not prepared at a home? By meal, I mean breakfast,	Number Don't know 77 ⌊_⌋⌊_⌋	D6

Participant Identification Numb ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋

CORE: Physical Activity

Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person.

Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. *[Insert other examples if needed]* In answering the following questions 'vigorous-intensity activities'

Question		Response	Code
Work			
52	Does your work involve vigorous-intensity activity that causes large increases in *[carrying or lifting heavy loads, digging or construction work]* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P 4*	P1
53	In a typical week, on how many days do you do vigorous-intensity activities as part	Number of days ⌊_⌋	P2
54	How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours : minutes ⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ hrs mins	P3 (a-b)
55	Does your work involve moderate-intensity activity, that causes small increases in breathing or heart rate such as brisk walking *[or carrying light loads]* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P 7*	P4
56	In a typical week, on how many days do you do moderate-intensity activities as	Number of days ⌊_⌋	P5
57	How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours : minutes ⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ hrs mins	P6 (a-b)
Travel to and from places			
The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship. *[Insert other examples if needed]*			
58	Do you walk or use a bicycle *(pedal cycle)* for at least 10 minutes continuously to get to and from places?	Yes 1 No 2 *If No, go to P 10*	P7
59	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from	Number of days ⌊_⌋	P8
60	How much time do you spend walking or bicycling for travel on a typical day?	Hours : minutes ⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ hrs mins	P9 (a-b)

CORE: Physical Activity, Continued			
Question		**Response**	**Code**
Recreational activities			
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure), *[Insert relevant*			
61	Do you do any vigorous-intensity sports, fitness or recreational *(leisure)* activities that cause large increases in breathing or heart rate like *[running or football]* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P 13*	P10
62	In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational *(leisure)* activities?	Number of days ⌊_⌋	P11
63	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?	Hours : minutes ⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ hrs mins	P12 (a-b)
64	Do you do any moderate-intensity sports, fitness or recreational *(leisure)* activities that cause a small increase in breathing or heart rate such as brisk walking, *[cycling, swimming, volleyball]* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P16*	P13
65	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational *(leisure)* activities?	Number of days ⌊_⌋	P14
66	How much time do you spend doing moderate-intensity sports, fitness or recreational *(leisure)* activities on a typical day?	Hours : minutes ⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋	P15 (a-b)

EXPANDED: Physical Activity			
Sedentary behaviour			
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent sitting at a desk, sitting with friends, traveling in car, bus, train, reading, playing cards or watching television, but do not include time spent sleeping. *[INSERT EXAMPLES]* *(USE*			
67	How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes ⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ hrs mins	P16 (a-b)

Participant Identification Numb ⌊_⌊_⌊_⌋ ⌊_⌊_⌊_⌋ ⌊_⌊_⌊_⌋

CORE: History of Raised Blood Pressure			
Question		**Response**	**Code**
68	Have you ever had your blood pressure measured by a doctor or other health worker?	Yes 1 No 2 *If No, go to H6*	H1
69	Have you ever been told by a doctor or other health worker that you have raised blood pressure or hypertension?	Yes 1 No 2 *If No, go to H6*	H2a
70	Have you been told in the past 12 months?	Yes 1 No 2	H2b

EXPANDED: History of Raised Blood Pressure			
71	Are you currently receiving any of the following treatments/advice for high blood pressure prescribed by a doctor or		
	Drugs (medication) that you have taken in the past two weeks	Yes 1 No 2	H3a
	Advice to reduce salt intake	Yes 1 No 2	H3b
	Advice or treatment to lose weight	Yes 1 No 2	H3c
	Advice or treatment to stop smoking	Yes 1 No 2	H3d
	Advice to start or do more exercise	Yes 1 No 2	H3e
72	Have you ever seen a traditional healer for raised blood pressure or hypertension?	Yes 1 No 2	H4
73	Are you currently taking any herbal or traditional remedy for your raised blood pressure?	Yes 1 No 2	H5

Participant Identification Numb ⌊_⌊_⌊_⌋ ⌊_⌊_⌊_⌋ ⌊_⌊_⌊_⌋

CORE: History of Diabetes			
Question		**Response**	**Code**
74	Have you ever had your blood sugar measured by a doctor or other health worker?	Yes 1 No 2 *If No, go to M1*	H6
75	Have you ever been told by a doctor or other health worker that you have raised	Yes 1 No 2 *If No, go to M1*	H7a
76	Have you been told in the past 12 months?	Yes 1 No 2	H7b

EXPANDED: History of Diabetes			
77	Are you currently receiving any of the following treatments/advice for diabetes prescribed by a doctor or other health		
	Insulin	Yes 1 No 2	H8a
	Drugs (medication) that you have taken in the past two weeks	Yes 1 No 2	H8b
	Special prescribed diet	Yes 1 No 2	H8c
	Advice or treatment to lose weight	Yes 1 No 2	H8d
	Advice or treatment to stop smoking	Yes 1 No 2	H8e
	Advice to start or do more exercise	Yes 1 No 2	H8f
78	Have you ever seen a traditional healer for diabetes or raised blood sugar?	Yes 1 No 2	H9
79	Are you currently taking any herbal or traditional remedy for your diabetes?	Yes 1 No 2	H10

Participant Identification Numb ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋

Step 2 Physical Measurements

CORE: Height and Weight			
Question		**Response**	**Code**
80	Interviewer ID	⌊_⌋⌊_⌋⌊_⌋	M1
81	Device IDs for height and weight	Height ⌊_⌋⌊_⌋ Weight ⌊_⌋⌊_⌋	M2a M2b
82	Height	in Centimetres (cm) ⌊_⌋⌊_⌋⌊_⌋.⌊_⌋	M3
83	Weight *If too large for scale 666.6*	in Kilograms (kg) ⌊_⌋⌊_⌋⌊_⌋.⌊_⌋	M4
84	**For women:** Are you pregnant?	Yes 1 *If Yes, go to M 8* No 2	M5
CORE: Waist			
85	Device ID for waist	⌊_⌋⌊_⌋	M6
86	Waist circumference	in Centimetres (cm) ⌊_⌋⌊_⌋⌊_⌋.⌊_⌋	M7
CORE: Blood Pressure			
87	Interviewer ID	⌊_⌋⌊_⌋⌊_⌋	M8
88	Device ID for blood pressure	⌊_⌋⌊_⌋	M9
89	Cuff size used	Smal 1 l 2 Mediu 3	M10
90	Reading 1	Systolic (mmHg) ⌊_⌋⌊_⌋⌊_⌋	M11a
		Diastolic (mmHg) ⌊_⌋⌊_⌋⌊_⌋	M11b
91	Reading 2	Systolic (mmHg) ⌊_⌋⌊_⌋⌊_⌋	M12a
		Diastolic (mmHg) ⌊_⌋⌊_⌋⌊_⌋	M12b
92	Reading 3	Systolic (mmHg) ⌊_⌋⌊_⌋⌊_⌋	M13a
		Diastolic (mmHg) ⌊_⌋⌊_⌋⌊_⌋	M13b
93	During the past two weeks, have you been treated for raised blood pressure with drugs (medication) prescribed by a doctor or	Yes 1 No 2	M14

EXPANDED: Hip Circumference and Heart Rate			
94	Hip circumference	in Centimeters (cm) ⌊_⌋⌊_⌋⌊_⌋.⌊_⌋	M15
95	Heart Rate		
	Reading 1	Beats per minute ⌊_⌋⌊_⌋⌊_⌋	M16a
	Reading 2	Beats per minute ⌊_⌋⌊_⌋⌊_⌋	M16b
	Reading 3	Beats per minute ⌊_⌋⌊_⌋⌊_⌋	M16c

CORE: Blood Glucose			
Question		**Response**	**Code**
96	During the past 12 hours have you had anything to eat or drink, other than water?	Yes 1 No 2	B1
97	Technician ID	⌊_⌋⌊_⌋⌊_⌋	B2
98	Device ID	⌊_⌋⌊_⌋	B3
99	Time of day blood specimen taken (24 hour clock)	Hours : minutes ⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ hrs mins	B4
100	Fasting blood glucose *Choose accordingly: mmol/l or mg/dl*	mmol/l ⌊_⌋⌊_⌋ . ⌊_⌋⌊_⌋ mg/dl ⌊_⌋⌊_⌋⌊_⌋ . ⌊_⌋	B5
101	Today, have you taken insulin or other drugs (medication) that have been prescribed by a doctor or other health worker	Yes 1 No 2	B6
CORE: Blood Lipids			
102	Device ID	⌊_⌋⌊_⌋	B7
103	Total cholesterol *Choose accordingly: mmol/l or mg/dl*	mmol/l ⌊_⌋⌊_⌋ . ⌊_⌋⌊_⌋ mg/dl ⌊_⌋⌊_⌋⌊_⌋ . ⌊_⌋	B8
104	During the past two weeks, have you been treated for raised cholesterol with drugs (medication) prescribed by a doctor or	Yes 1 No 2	B9

EXPANDED: Triglycerides and HDL Cholesterol			
105	Triglycerides *Choose accordingly: mmol/l or mg/dl*	mmol/l ⌊_⌋⌊_⌋ . ⌊_⌋⌊_⌋ mg/dl ⌊_⌋⌊_⌋⌊_⌋ . ⌊_⌋	B10
106	HDL Cholesterol *Choose accordingly: mmol/l or mg/dl*	mmol/l ⌊_⌋ . ⌊_⌋⌊_⌋ mg/dl ⌊_⌋⌊_⌋⌊_⌋ . ⌊_⌋	B11

World Health Organization

Participant Identification Numb ⌊_⌋_⌋_⌋ ⌊_⌋_⌋_⌋ ⌊_⌋_⌋_⌋

APPENDIX 3: The Pittsburgh Sleep Quality Index Questionnaire

Name_____Date_____

Sleep Quality Assessment (PSQI)

INSTRUCTIONS:

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? _____

2. How long (in minutes) has it taken you to fall asleep each night? _____

3. What time have you usually gotten up in the morning? _____

4. A. How many hours of actual sleep did you get at night? _____

   B. How many hours were you in bed? _____

5. During the past month, how often have you had trouble sleeping because you	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
A. Cannot get to sleep within 30 minutes				
B. Wake up in the middle of the night or early morning				
C. Have to get up to use the bathroom				
D. Cannot breathe comfortably				
E. Cough or snore loudly				
F. Feel too cold				
G. Feel too hot				
H. Have bad dreams				
I. Have pain				
J. Other reason (s), please describe, including how often you have had trouble sleeping because of this reason (s):				
6. During the past month, how often have you taken medicine (prescribed or “over the counter”) to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
9. During the past month, how would you rate your sleep quality overall?	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)

APPENDIX 4: Centre for Epidemiologic Studies Depression (CES-D) Questionnaire

	During the past week:	Rarely or none of the time (less than 1 day)	Some or a little of the time (1-2 days)	Occasionally or a moderate amount of time (3-4 days)	Most or all of the time (5-7 days)
1.	I was bothered by things that usually don't bother me.				
2.	I did not feel like eating; my appetite was poor.				
3.	I felt that I could not shake off the blues even with help from my family or friends.				
4.	I felt I was just as good as other people.				
5.	I had trouble keeping my mind on what I was doing.				
6.	I felt depressed.				
7.	I felt that everything I did was an effort.				
8.	I felt hopeful about the future.				
9.	I thought my life had been a failure.				
10.	I felt fearful.				
11.	My sleep was restless.				
12.	I was happy.				
13.	I talked less than usual.				
14.	I felt lonely.				
15.	People were unfriendly.				
16.	I enjoyed life.				
17.	I had crying spells.				
18.	I felt sad.				
19.	I felt that people disliked me.				
20.	I could not get going.				

APPENDIX 5: The Short-Form 12 (SF-12) Questionnaire

General Health Subdomain

In general, would you say your health is excellent, very good, good, fair, or poor?

1) Excellent

2) Very good

3) Good

4) Fair

5) Poor

Physical Functioning Subdomain

How does your health now limit you in moderate activities, such as moving a table, doing house chores? Would you say you are limited a lot, a little or not at all?

1) Yes, limited a lot

2) Yes, limited a little

3) No, not limited at all

How about climbing several flights of stairs? Would you say your health limits you a lot, a little, or not at all?

1) Yes, limited a lot

2) Yes, limited a little

3) No, not limited at all

Role Functioning (Physical) Subdomain

Thinking about the past four weeks, have you accomplished less than you would like as a result of your physical health?

1) Yes

2) No

During the past four weeks, were you limited in the kind of work or other activities you could do as a result of your physical health?

1) Yes

2) No

Bodily Pain Subdomain

During the past four weeks, how much did pain interfere with your normal work including both work outside the home and housework?

1) Extremely

2) Quite a bit

3) Moderately

4) A little bit

5) Not at all

Vitality Subdomain

How much of the time during the past four weeks did you have a lot of energy? Would you say (read responses)?

1) None of the time

2) A little of the time

3) Some of the time

4) Good bit of the time

5) Most of the time

6) All of the time

Role Functioning (Emotional) Subdomain

In the past four weeks, did you accomplish less than you would like as a result of an emotional problem, such as feeling depressed or anxious?

1) Yes

2) No

During the last four weeks, did you have trouble doing work or other activities as carefully as usual as a result of an emotional problem, such as feeling depressed or anxious?

1) Yes

2) No

Mental Health Subdomain

How much of the time during the past four weeks have you felt calm and peaceful? Would you say (read responses)?

1) None of the time

2) A little of the time

3) Some of the time

4) Good bit of the time

5) Most of the time

6) All of the time

How much of the time during the past four weeks have you felt downhearted and blue? (If necessary, read responses)

1) All of the time

2) Most of the time

3) Good bit of the time

4) Some of the time

5) A little of the time

6) None of the time

Social Functioning Subdomain

During the last four weeks, how much of the time has your physical health or emotional problems interfered with your social activities, like visiting with friends, relatives etc.?

(If necessary, read responses)

APPENDIX 6: Ethics approval document from Warwick Medical School, University of Warwick

12th May 2014

Warwick Medical School

PRIVATE
Dr Chidozie Nduka
8 Branstree Drive
Coventry
CV6 6GB

Dear Chidozie,

Study Title and BSREC Reference: *Highly Active Antiretroviral Therapy and Cardiovascular Risk Factor Profiles of HIV-Infected Subjects in Nigeria: A Cross-sectional Study* REGO-2014-711

Thank you for submitting your revisions to the above-named project to the University of Warwick Biomedical and Scientific Research Ethics Sub-Committee for Chair's Approval.

I am pleased to confirm that I am satisfied that you have met all of the conditions and your application meets the required standard, which means that full approval is granted and your study may commence.

I take this opportunity to wish you success with the study and to remind you any substantial amendments require approval from the committee before they can be made. Please keep a copy of the signed version of this letter with your study documentation.

Yours sincerely,

PP

David Davies
Chair
Biomedical and Scientific
Research Ethics Sub-Committee

Biomedical and Scientific Research Ethics Subcommittee
A010 Medical School Building
Warwick Medical School,
Coventry, CV4 7AL
Tel: 02476-151875
Email: BSREC@Warwick.ac.uk

THE UNIVERSITY OF WARWICK

APPENDIX 7: Ethics approval document from the Benue State University Teaching Hospital

BENUE STATE UNIVERSITY TEACHING HOSPITAL
MAKURDI - NIGERIA.

BOARD CHAIRMAN
Prof Peter O. Obekpa
MB, FRCS, FMCS, FWACS, FICS

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CHIEF MEDICAL DIRECTOR
Prof A. O. Malu (OON)
MB. FMCP, FWACP

CHAIRMAN MEDICAL ADVISORY COMMITTEE
Dr. Hembah-Hilekaan S. K.
MBBS. FIIA, FWACS

E-mail: bsuth_estab@yahoo.com

Ref: REGISTRATION NUMBER: **NHREC/08/11/2013B** **Date:** 28th August, 2014.

HEALTH RESEARCH ETHICS COMMITTEE

Dr. Chidozie Nduka

The University of Warwick

Coventry

UK.

Research Title: Antietroviral Therapy and Cardiovascular Risk Factor Profiles of HIV-Infected Subjects in Nigeria: A Cross-Sectional Study.

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

This is to inform you that the research described in the submitted protocol, the consent forms and other participant information material have been reviewed and given **full approval** by the Health Research Ethics Committee during its 10th meeting held on 27th August, 2014.

This approval dates from August 28th 2014 to August 28th 2015. If there is delay in starting the research, please inform the HREC so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside these dates. All informed consent forms used in this study must carry the HREC assigned number and the duration of HREC approval of the study.

The **National Code for Health Research Ethics** requires you to comply with all institutional guidelines, rules and regulations and with the tenet of the Code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit to your research site without previous notification

28/8/14

Dr. Audu Onyemocho

Chairman.

APPENDIX 8: Participant information leaflet

THE UNIVERSITY OF WARWICK

Provisional Study Title:	ANTIRETROVIRAL THERAPY AND CARDIOVASCULAR DISEASE RISK FACTOR PROFILES OF HIV-INFECTED PATIENTS IN NIGERIA: A CROSS-SECTIONAL SUDY
Investigator:	DR. CHIDOZIE NDUKA

Introduction

You are invited by the University of Warwick to take part in a research study. Before you decide, you need to understand why the research is being done and what it would involve for you. Please take the time to read the following information carefully. Talk to others about the study if you wish.

(Part 1 tells you the purpose of the study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study)

Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

PART 1

What is the study about?

HIV-infected patients have to take their HIV drugs in order to live longer. However, there are now concerns that these HIV drugs can cause heart problems after a long period of use. So we would like to compare the numbers of patients who are at risk of developing heart disease or stroke between HIV-infected patients who have been receiving HIV drugs and HIV-infected patients who are not yet required to receive their HIV drugs. It is important for us to determine if this association exists so that we can improve your care.

Why have I been asked to take part?

You have been asked to take part in this study because you have been living with HIV for some time now. Only HIV-infected patients attending this clinic are eligible to participate in this study and we would like to assess your risk of developing heart disease or stroke because HIV and HIV drugs have the potential for complications or side effects that affect

Do I have to take part?

It is your decision to take part (or not) in this study. We will describe the study and go through this information sheet, which we will give you to keep. If you choose to participate, we will ask you to sign a consent form to confirm that you have agreed to take part. You will be free to withdraw at any time, without giving a reason and this will not affect you or your circumstances in any way.

What will happen to me if I take part?

After you decide to take part, we will ask you a series of questions and run some tests for you at no cost. The tests will include measuring the glucose and fat levels in your blood. We will also measure your blood pressure. As regards the questions, we would it if you provided us with all the answers; however, please be aware that it is within your rights to refuse to answer a particular question. However, we need to run the tests if you decide to take part in this research study.

What are the possible disadvantages, side effects, risks, and/or discomforts of taking part in this study?

The questionnaires might take a while to answer, but I will guide you through all of them. The tests will involve a small needle prick on one of your fingertips to get the blood samples. You might experience a slight sudden pain, which is only produced by the jab. The pain becomes a dull ache that subsides within a few minutes. You might also experience some slight bruising at the jabbed fingertip, but this also resolves veryshortly. The jabbed fingertip might be gently milked to let down more blood onto the test strip. However, no more than two drops of blood (0.1mL) is needed for the tests and there would be no need for storage. The procedure is not known to be life-threatening or constitute severe complications in adults; however, it will be carried out by experienced staff. We have also provided sufficient numbers of lancets to ensure that no lancet is used twice or shared. You might also experience some slight discomfort from inflating the cuff during

What do I gain from taking part?

You get to know whether or not you are at a higher risk than normal of developing heart disease or stroke in the future. If you are, the information will be indicated in your medical record for your doctor to see and take necessary action. However, there should be no cause for worry as the risk is not immediately life-threatening and can be modified.

Expenses and payments

Transport costs will be subsidized for those patients who return to participate in the study in advance of their next follow-up appointment at the clinic.

What will happen when the study ends?

After we have gathered all the information that is needed, we will analyse them at the University of Warwick. We will also send a summary of the results to the clinic.

Will my taking part be kept confidential?

Yes. We will follow strict ethical and legal practice and all information about you will be handled in confidence. We will also not be requiring names. Your formation will be kept safe in a secure online location at all times, accessed only using the researcher's University of Warwick user account.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm that you might suffer will be addressed. Detailed information is given in Part 2.

This concludes Part 1.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART 2

Who is organising and funding the study?

This study is being conducted in fulfilment of the degree of PhD at the University of Warwick, United Kingdom.

What will happen if I don't want to carry on being part of the study?

Participation in this study is entirely voluntary. Refusal to participate will not affect you in any way. If you decide to take part in the study, you will need to sign a consent form, which states that you have given your consent to participate.

If you agree to participate, you may nevertheless withdraw from the study at any time without affecting you in any way.

You have the right to withdraw from the study completely and decline any further contact by study staff after you withdraw.

What if there is a problem?

This study is covered by the University of Warwick's insurance and indemnity cover. If you have an issue, please contact Jo Horsburgh (details below).

Who should I contact if I wish to make a complaint?

Any complaint about the way you have been dealt with during the study or any possible harm you might have suffered will be addressed. Please address your complaint to the person below, who is a Senior University of Warwick official entirely independent of this study:

Jo Horsburgh
Deputy Registrar
Deputy Registrar's
Office University of
Warwick Coventry, UK,

What will happen to the results of the study?

The results of the study will be summarized and sent to the clinic to be forwarded to participants who require a copy. We would also like to present the results in conferences as well as publish them in key medical journals.

Who has reviewed the study?

This study has been reviewed and given favourable opinion by the University of Warwick's Biomedical and Scientific Research Ethics Committee (BSREC): (*BSREC number: REGO-2014-711*).

What if I want more information about the study?

If you have any questions about any aspect of the study or your participation that is not answered by this participant information leaflet, please contact any of the following people:

Dr. Chidozie U. Nduka; C.U.Nduka@warwick.ac.uk;
+44(0)7472730268 Dr. Saverio Stranges; S.Stranges@warwick.ac.uk;
+44(0)2476151153
Dr. Olalekan A. Uthman; Olalekan.uthman@warwick.ac.uk; +44(0)2476573163

Thank you for taking the time to read this participant information leaflet.

APPENDIX 9: Participant consent form

THE UNIVERSITY OF WARWICK

BIOMEDICAL AND SCIENTIFIC RESEARCH ETHICS COMMITTEE CONSENT FORM

Study Number: REGO-2014-711

Patient Identification Number for this study:

Provisional Title of Project: ANTIRETROVIRAL THERAPY AND CARDIOVASCULAR DISEASE RISK FACTOR PROFILES OF HIV-INFECTED PATIENTS IN NIGERIA

Name of Researcher(s): Dr. Chidozie Nduka; Dr. Saverio Stranges; Dr. Olalekan Uthman; Dr. Peter Kimani, Professor Abraham O. Malu.

Please initial all boxes

1. I confirm that I have read / had the information leaflet read to me. I understand the information contained on the leaflet dated **[**DATE**]** for the above study. I have had the opportunity to consider these information, asked questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care being affected. ☐
3. I understand that a sample of my blood would be needed as part of this study. I also understand that the procedure for obtaining this sample may be associated with some discomfort, and I give consent. ☐
4. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from The University of Warwick and regulatory authorities involved in policy formulation and implementation. I give permission for these individuals to have access to my records. ☐
5. I agree to take part in the above named study. ☐
6. I understand that my physician will be informed of my participation in this study. ☐

_____	_____	_____
Name of Participant	Date	Signature/thumb print

_____	_____	_____
Name of Person taking consent	Date	