THE EFFECTS OF HIV AND ART ON SERUM LIPIDS AMONG ADULTS IN AGINCOURT IN 2015

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Declaration

I declare that this research report is my own work. It is being submitted in partial fulfillment of the requirements for the degree of Master of Science in Epidemiology in the field of Epidemiology & Biostatistics at the University of the Witwatersrand, Johannesburg. This research has not been submitted previously for any degree or examination to any other institution.

Dr. Engelbert Adamwaba Nonterah

Nineteenth day of June 2017 in Johannesburg

Dedication

I dedicate this work to my family: Mrs. Cynthia Nonterah and my son Wedaga Cyrus Nonterah who endured my absence while I was away in school. I do appreciate their sacrifices, support and prayers.

Also to my parents Mr. Henry Alexis Navoro Nonterah and Madam Faustina Nabonawura Tankia as well as my siblings: Josephine Kawiah Nonterah, Margaret Anuyire Nonterah, Mary-Esther Yagade Nonterah and Esmond Wedam Nonterah. Thanks for your prayers and words of encouragement.

Abstract

Background: The burden of HIV infection is still high in South Africa. However, the use of ART has greatly improved treatment outcomes and survival. People infected with HIV and receiving ART are therefore living longer but with a likely increase in their cardiometabolic risk. Both HIV infection and anti-retroviral drugs have been shown to affect serum lipid levels and this may be among the reasons for the increased cardiometabolic risk in these subjects. The aim of this study was therefore to characterize the principal determinants of lipid levels in a large rural South African population with a high prevalence of HIV infection in which an array of factors that possibly modulate serum lipid levels had also been measured.

Materials and methods: Data for this secondary analysis are drawn from a population-based cross-sectional study: the HAALSI/AWI-Gen collaborative study conducted in the Agincourt sub-district of the Mpumalanga province. 2110 adults 40+ years being monitored by the Agincourt health and socio-demographic surveillance system were randomly selected and recruited, after giving informed consent, between 2013-2016. Pretested questionnaires were used to collect personal, household, socio-demographic, behavioral, dietary, physical activity and self-reported health status. Anthropometric measurements were also conducted. Multivariable linear and logistic regression analyses were used to determine factors associated with serum lipid levels and dyslipidemia, respectively.

Results: Results are presented for 2110 participants in this secondary analysis of which 60.3% were women with a mean population age of 58.54 ± 10.91 years. The HIV prevalence was 16.16% and did not differ substantially between men and women. Factors associated with total cholesterol level included age (unstandardized beta [95% CIs] was: 0.02 [0.01, 0.03]; p=0.014), male gender (-0.31 [-0.57, -0.05]; p=0.019), diabetes (0.31 [0.01, 0.61]; p=0.039), alcohol consumption (0.25 [0.02, 0.48]; p=0.038) and BMI (0.02 [0.01, 0.04]; p=0.030). Factors associated with triglycerides included age (0.01 [0.01, 0.03]; p=0.003), male gender (-0.09 [-0.19, 0.01]; p=0.053), diabetes (0.27 [0.13, 0.40]; p<0.0001), BMI (0.01 [0.01, 0.03]; p=0.044), hip circumference (-0.01 [-0.02, -0.01]; p=0.001) and waist circumference (0.01 [0.01, 0.02]; p<0.0001). Factors associated with HDL-C level included age (-0.01 [-0.01, 0.01]; p=0.055), male gender (-0.14 [-0.26, -0.02]; p=0.018), receiving ART (0.17 (0.04, 0.31**);** p=0.038), alcohol consumption (0.19 [0.07, 0.30]; p=0.002), waist circumference (-0.01 [-0.01, -0.001]; p=0.001) and visceral adipose tissue (-0.03 [-0.04, -0.01]; p=0.002). Age (0.02 (0.01, 0.03); p=0.005),

male gender (-0.22 (-0.43, -0.01); p=0.044) and waist circumference (0.01 (0.01, 0.02); p<0.0001) were all associated with LDL-C levels. Being HIV+ and ART naive was associated with a higher risk of dyslipidemia (odds ratio [95% CIs] was 3.79 [1.27, 11.30]; p=0.032) compared to HIV negative participants. Other factors associated with dyslipidemia included being overweight (1.66 [1.20, 2.30] p=0.002) and obese (OR 1.85 [1.02, 3.35]; p=0.0004) and increased waist circumference (OR 1.02 [1.01, 1.03]; p<0.0001).

Discussion and conclusion: We have demonstrated a high prevalence of HIV in an older population of rural South Africa, which mirrors the typical epidemiology of the epidemic in southern and eastern African regions. Our data suggest that HIV/ART status mainly influences HDL-C levels with ART use associated with higher HDL; and that untreated HIV infection can be linked to a greater risk of dyslipidemia. Dyslipidemia in the study population is driven by prevailing traditional cardiovascular risk factors such as obesity and diabetes. This data suggests that high ART coverage may reduce atherogenic risk and that lifestyle interventions to reduce the risk of obesity and diabetes are essential.

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1.0 CHAPTER 1: INTRODUCTION

This chapter gives a brief background to the study by summarizing current knowledge as well as gaps in this knowledge. Presented here also is the burden of HIV/AIDS and antiretroviral therapy (ART) coverage in sub-Saharan Africa and South Africa. The chapter further describes the burden of cardiometabolic diseases as well as risk factors among HIV/AIDS patients who use or do not use ART.

This chapter gives a review of published literature on normal lipid and cholesterol metabolism and how HIV and ART independently affect serum lipids. We also summarize factors associated with serum lipids and dyslipidemia and their subsequent influence on cardiovascular risk. A brief description of the various methods of assessing dyslipidemia is included in this chapter. The chapter also includes the statement of the problem addressed by the study and a justification for the study. We conclude this chapter with the research question addressed the aim and objectives of the study.

1.1 Background to the study

Dyslipidemia is associated with an increased risk of cardiometabolic disease (1) among HIV infected patients and the general population (2, 3). ART often exacerbates it among people living with HIV (PLWH) (4, 5). While sub-Saharan Africa continues to bear a huge burden of HIV/AIDS and tuberculosis (6), non-communicable diseases (NCDs) are also on the increase (7). The Eastern and Southern Africa regions alone contributed nearly half (46%) of people living with HIV (PLWH) globally in 2015 (8).

Though the number of PLWH is still high (and increasing), AIDS-related mortalities have decreased by 45% since the peak in 2005 and new cases are declining globally (8). Similarly the national prevalence of HIV in South Africa has declined from 12.2% in 2012 (9) to 11.2% by mid-2015 (10). There has also been an observed increase in access and use of antiretroviral therapy (ART) with the current global estimate of 54% among PLWH (8). This global situation reflects what has been experienced by South Africa, which continues to suffer a huge burden of the HIV epidemic (8-10).

The increase in ART coverage has led to a decrease in incidence of opportunistic infections and resulted in long-term survival among PLWH (11). Urbanization, changing lifestyles and changing age and sex structure of most African countries due to the current epidemiological transition, has also contributed to a rise in the proportion of older people in the population (12, 13).

Disease patterns are gradually shifting from dominantly infectious to non-infectious (or a mix of the two) as a major source of morbidity and mortality (2, 14). Thus, a threefold increase in non-communicable diseases has been observed for low and middle income countries (LMIC) with Ghana, Nigeria and South Africa experiencing a rise in prevalence of chronic diseases in the midst of a continuing threat of infectious diseases (1, 12).

In South Africa, 41% of all deaths were attributed to non-communicable diseases according to mortality trends from the second South African National Burden of Disease study (15).

The prevailing risk factors associated with NCDs include smoking, alcohol consumption, obesity, unhealthy diet, inadequate physical activity, psychological factors and dyslipidemia (2, 7, 16). While the high-income countries have the same prevailing risk factors, there is a decline in NCDs compared to sub-Saharan Africa. This is largely due to effective population and individual level interventions (1).

Atherogenic dyslipidemia has been implicated as a cause of cardiovascular diseases (CVDs) in both the general population and among PLWH (3). Studies from high-income countries have implicated HIV infection itself, the use of ART as well as exposure to the prevailing classical CVD risk factors experienced by the general population, as the causes of increased atherogenic dyslipidemia among PLWH (17, 18).

However, comparative population-based epidemiological data on the variations in lipid levels and prevalence of dyslipidaemia among PLWH and the general population is scarce in sub-Saharan Africa (4) as are any in-depth studies on the factors causing dyslipidemia.

1.2 Literature review

1.2.1 Epidemiology

Globally, PLWH have increased from 33.3 million in 2010 to 36.7 million people in 2015 (8) . Sub-Saharan African contributes significantly to the global burden of HIV with South Africa having the highest prevalence of HIV (8). The 2015 mid-year prevalence study in South Africa reported an estimated overall prevalence of approximately 11.2% (6.19 million PLWH in 2015) within the population (10). Adults aged 15-49 years had a higher estimated HIV prevalence of 16.6% (10).

Though the prevalence is still high, South Africa has experienced a 60% reduction in new cases of HIV from 2008 to 2012 (9). This can be attributed to effective public health interventions including an increase in ART coverage among PLWH from 16.6% to 31.2% (9).

Globally, HIV-related mortalities are generally decreasing while diseases of lifestyle are increasingly becoming a major cause of morbidity and mortality among PLWH (3). Data on trends in mortality across Africa are generally scarce. A study in Uganda observed a sustained 11 years of decline in the trends of HIV-related mortalities since the introduction of highly active antiretroviral therapy in 2004 (11). Similarly, mortality from opportunistic infection among HIV patients has declined since the scale-up of ART in 2004 in South Africa (14).

Globally, NCDs accounted for 63% of deaths (48% due to CVDs) in 2015 and projected to increase to 75% by 2030 in the general population (1). In South Africa, it is estimated that 40% of all deaths in 2008 were due to NCDs with the leading reported cause being CVD (15). These changes result from the ongoing epidemiological and demographic transition across Africa (12, 13). Life expectancy has generally increased making diseases of lifestyle the major contributor to morbidity and mortality.

PLWH are therefore living longer and experiencing the normal ageing process. This renders them prone to the traditional cardiovascular risk factors confronting the general population (3) as well as HIV-specific and ART-related mechanisms that appear to increase cardiovascular risk (19, 20).

1.2.2 Normal cholesterol and lipid metabolism

Lipid metabolism involves the breakdown and storage of endogenous (produced in liver and peripheral tissues) and exogenous (dietary sources absorbed from the gut) fat in the liver (see Figure 1.1). The liver synthesises approximately 1g of cholesterol whilst the human diet accounts for approximately 400mg daily (21).

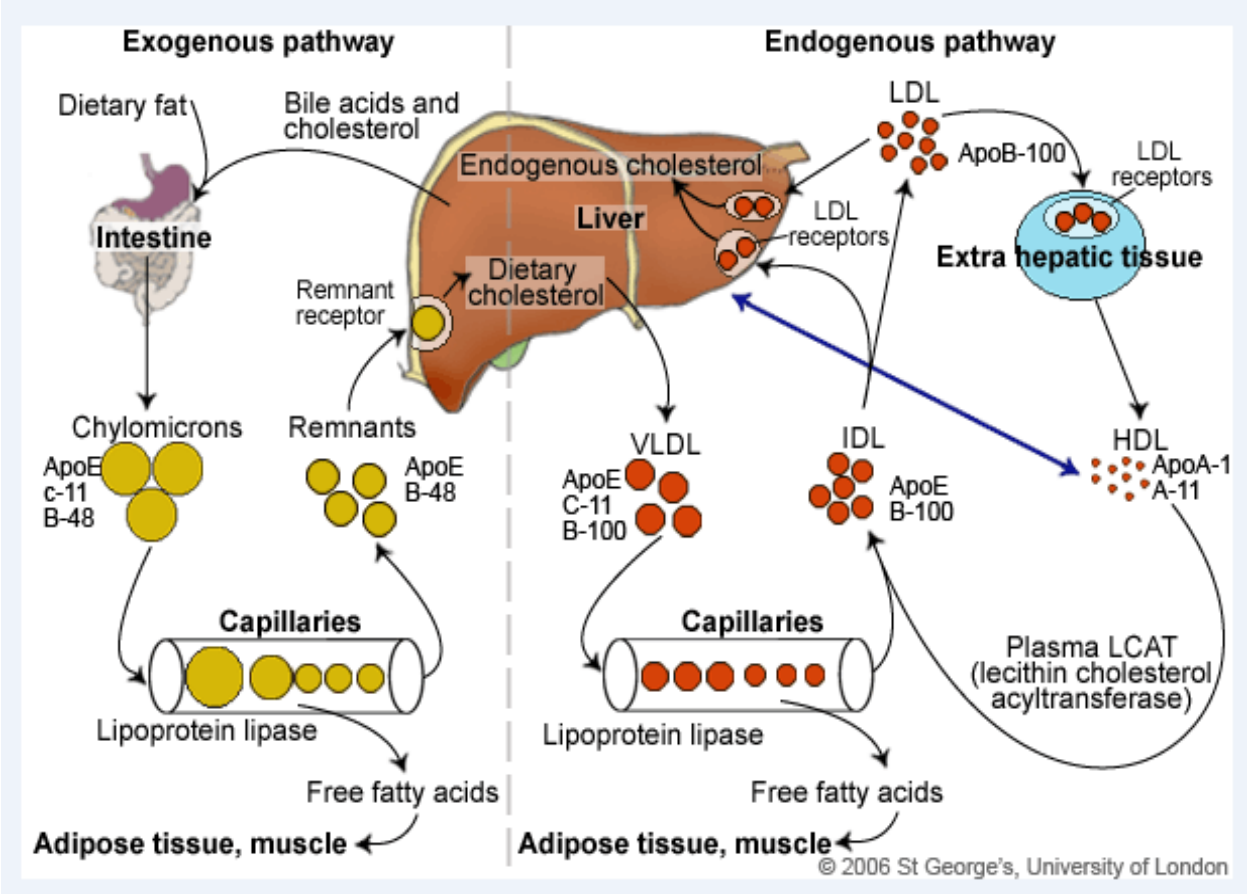

Figure 1.1: Lipid and cholesterol metabolism **(Courtesy: https://www.google.co.za and http://www.sgul.ac.uk)**

Not only does the liver synthesize cholesterol for export to other cells, but it also removes cholesterol from the body by converting it to bile salts in which form it can be eliminated in the faeces. Furthermore, the liver synthesizes the various lipoproteins involved in transporting cholesterol and other lipids throughout the body (22).

Endogenous lipids are derived from lipogenesis (fatty acid and triglyceride synthesis) in the liver. In this process, acetyl-coA, an intermediate product of carbohydrate metabolism is converted to fatty acids (21). The synthesised fatty acids are then secreted from the liver into the bloodstream as very low-density lipoprotein (VLDL), which is formed from triglycerides and cholesterol esters. The VLDL then functions to deliver endogenously derived lipids to peripheral tissues (22).

The VLDL is hydrolysed by lipoprotein lipase in the peripheral tissues, particularly skeletal muscle and adipose tissue to release free fatty acids and glycerol, with the free fatty acids been taken up into the tissue. This hydrolysis of the VLDL particles gives rise to intermediate density lipoprotein (IDL). The IDL is then cleared from the circulation by the liver or incorporated into LDL. The LDL particles contain a core of cholesterol esters and a smaller amount of triglycerides.

The LDL is then used as a source of cholesterol for cells being internalized by hepatic and non-hepatic tissues. In the liver LDL is converted into bile acids and is secreted into the intestines and in the non-hepatic tissues it is used in hormone production, cell membrane synthesis or stored.

The excess LDL in the circulation can also become oxidised and this oxidised LDL is taken up by macrophages inducing the formation of foam cells. These cells are the initiators and principal components in atherosclerotic plaque formation (21, 22). Most of the dietary cholesterol exists as free sterol while 10-15% exists as cholesterol esters. The latter is hydrolysed by cholesterol esterase in the gut to release free cholesterol for absorption (21).

The resultant non-esterified cholesterol is then incorporated into bile acid micelles together with triglycerides, phospholipids, ionized fatty acids, 2-monoacylglycerol and lysophospholipids (21). The mixed micelle thus formed is then transported to the brush border of enterocytes where it is absorbed. Within the enterocytes further triglycerides and cholesterol then combine with the mixed micelle to form chylomicrons which enter into circulation and travel to peripheral tissues (21, 22).

In the peripheral tissues, free fatty acids are released from the chylomicrons by lipoprotein lipase to be used as energy or converted to triglyceride and stored in adipose tissue. Remnants are then used for the formation of VLDL particles in the liver (22).

HDL-C is a key mediator in the reverse cholesterol transport pathway (see Figure 1.2) by delivering cholesterol from macrophages in the arterial wall to the liver where it is processed for excretion from the body. In this pathway, HDL-C also transports cholesterol from the intestines to the liver. Lipid-poor Apo A-1 is secreted by the liver and intestinal cells into the circulation.

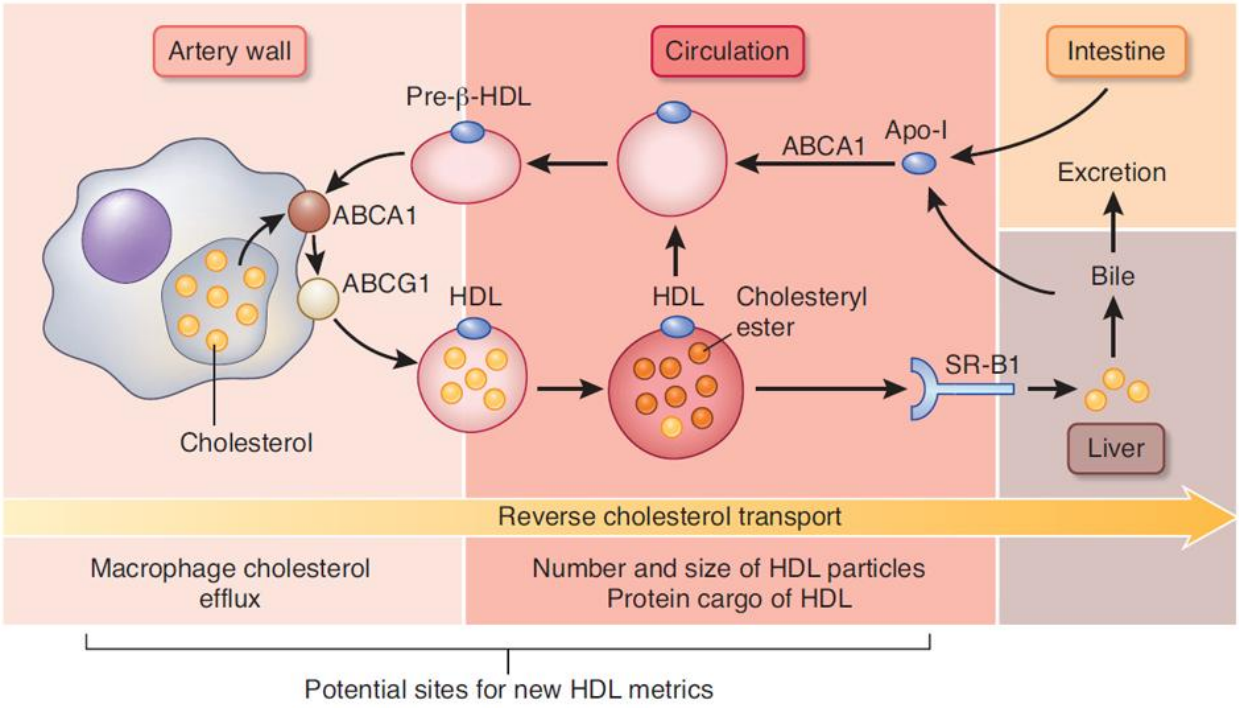

Figure 1.2: Mechanism of reverse cholesterol transport (source: **https://www.google.co.za)**

The Apo A-1, which makes up 70% of protein in HDL-C, is responsible for the anti-atherosclerotic properties of HDL-C. Apo A-1 interacts with ABCA1 to develop nascent HDL-particles, which accumulate more cholesterol from foam cells in the arterial wall.

The nascent and later matured HDL-C thus facilitates efflux of cholesterol from peripheral macrophages enabling reverse cholesterol transport (see Figure 1.2). The mature HDL-C delivers cholesterol to the liver through either the direct or the indirect pathway (23, 24).

The HDL-C interacts with the SR-B1 receptor on the liver via the direct pathway allowing delivery of cholesterol to the liver. The resulting lipid-poor HDL-C particle then re-enters circulation where it can repeat the process of reverse cholesterol transport.

In the indirect pathway, HDL-C interacts with the cholesterol ester transfer protein (CETP). This CETP protein facilitates the exchange of cholesteryl esters for triglycerides between HDL and VLDL or LDL-C particles (25). The LDL particles may remain in circulation or interact with the LDL-receptor on liver cells where LDL particles deposit the cholesterol ester for removal (25).

After cholesterol is delivered to the liver by either the direct or the indirect pathway, it is secreted into bile and carried to the intestines. The net effect of the reverse cholesterol transport pathway is the removal of cholesterol from plaques and the potential reduction of cardiovascular disease (23).

1.2.3 Dyslipidemia

PLWH often present with deranged lipid profiles due to abnormalities in lipid metabolism (2). Current literature on the aetiology of this observation is not clear (26). Several possible theories have been put forward to explain this observation. Thus, HIV infection itself and use of ART among HIV infected patients have independently been implicated as a cause of changes in lipid levels (18, 27, 28).

This association however, is potentially influenced by many factors that independently can result in abnormal serum lipidemia (26). The prevailing traditional risk factors, chronic inflammation resulting from HIV and the effect of drug metabolites as well as drug interactions have all been implicated as contributors to serum lipidemia (1, 19, 29).

Several other contributory factors include nutritional deficiencies from HIV infection, opportunistic infections, drug-induced cardio-toxicity and prolonged immune suppression (26).

1.2.4 HIV-associated dyslipidemia

Before the introduction of HAART, deranged lipid profiles were observed and described among HIV infected people (26). Early studies showed that patients infected with HIV type 1 (HIV-1)

when compared to uninfected patients had elevated levels of serum triglycerides (TGs) and decreased levels of serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) (18, 26).

Recent studies in Japan and other high-income countries among HIV-positive patients but antiretroviral therapy-naïve with no prior history of ART use confirmed the above observation (26, 30). The Japanese study demonstrated that HIV infection and socio-demographic factors independently influence serum lipid levels. The study further documented low HDL-C and high TGs are the predominant lipid abnormalities among HIV infected patients who were ART naïve (26).

Contrary to the above findings, hospital-based studies from sub-Saharan Africa found a decrease in serum LDL-C, HDL-C and triglycerides among HIV positive ART naïve patients compared to HIV patients on ART (31, 32). However, these studies did not take into account a wide range of confounding variables.

Possible mechanism: Serum viremia in early HIV-1 infection results in systemic inflammatory response and activation of the immune system. Lipoprotein lipase (LPL) and hepatic lipase levels decrease resulting in decreased TGs concentration and clearance. Hepatic synthesis of very low-density lipoprotein-cholesterol (VLDL) increases (33). Triglyceride concentration and clearance is reported to correlate strongly with overproduction of interferon-alpha (IFN-α) and other inflammatory cytokines such as tissue necrosis factor alpha (TNF-α) (33).

These pro-inflammatory mediators promote lipid peroxidation, disturb FFA metabolism and suppress hormone-mediated lipolysis (33-35). Activity of cholesterol ester transfer protein (CETP), which usually transfers cholesterol from HDL-C to apolipoprotein-B, is elevated in severe HIV viremia causing elevated TGs. The high activity of CETP in HIV infection however results in low HDL-C (19, 27, 29, 33).

The associated decrease in serum HDL-C levels is often described as a highly atherogenic state and thus increases cardiovascular risk (33). The fall in cholesterol levels observed with HIV infection may also occur because cholesterol plays a critical role in the replication of HIV-1 (35).

1.2.5 ART-associated dyslipidemia

The current goal in HIV case management is to achieve viral suppression using ARTs (3). Since the introduction of ART in the 1990's, prognosis among HIV patients has improved drastically (18, 32, 33). This has led to significant improvements in the quality and duration of life of most infected patients.

Associated with these gains however, are clinical and laboratory evidence of a rise in metabolic disorders among patients receiving ART (33, 36). The evidence points to the fact that ART is associated with lipodystrophy syndrome which is characterized by dyslipidemia, insulin resistance, fat redistribution to the visceral adipose tissue depot resulting in central obesity and to the nuchal region resulting in typical buffalo hump (cervical pad accumulation of fat) (33, 37). Other associated pathologies documented include metabolic bone disease (osteopenia and osteoporosis) and lactic acidosis (33).

Typically, ART associated dyslipidemia is characterized by raised serum triglycerides, total cholesterol and low-density lipoprotein (LDL-C), and a decrease in serum high-density lipoprotein (HDL-C) (33). Other molecules affected are very low-density lipoproteins (VLDL-C) and apolipoprotein B (apoB), both of which tend to be raised in the serum. These changes to the serum lipid profile increase cardiovascular risk in PLWH.

These metabolic changes associated with the use of ART are largely dependent on the drug regimen used (30). Nucleotide reverse transcriptase inhibitors (NRTIs) such as stavudine (d4T), lamivudine (3TC) and zidovudine (AZT) are reported to account for ART-related toxicity and metabolic changes (30). Unfortunately, due to cost, d4T and AZT were largely used across Africa as part of the first line regimen since the introduction of ARTs in 2004.

However, following reports of the severe toxicity and adverse metabolic outcomes, d4T has since being withdrawn (38) while AZT is still widely used as part of the first line regimen. Some of the protease inhibitors (PIs) such as ritonavir (RTV), lopinavir (LPV) and atazanavir (ATV), currently used as second line are also known to cause dyslipidemia (33, 34).

Current first line regimen recommended by the South African HIV physicians association includes a combination of one non-nucleoside reverse transcriptase inhibitor (NNRTI) such as

nevirapine (NVP) and efavirenz (EFV) and two suitable nucleotide reverse transcriptase inhibitors (one of which is zidovudine) (36).

Dyslipidemia among patients on newer antiretroviral agents such as etravirine (NNRTI), raltegravir (protease integrase strand transfer inhibitor), maraviroc (CCR5 antagonist), apricitabine or elvucitabine (NRTI) and bevirimat (maturation inhibitor) have also been reported from high-income countries (39, 40).

A study in Cape Town, South Africa among HIV patients documented elevated levels of triglycerides, LDL-C and TC among patients on ART compared to ART naïve patients. However HDL-C levels were lower in both patients on ART and ART-naive patients when compared to HIV negative patients (32).

In comparison, a study in Uganda showed high HDL-C in patients receiving ART, and TC and LDL-C levels were also observed to be high (41). Another longitudinal study in Johannesburg, South Africa showed that initiation of ART was associated with increased TC, triglycerides, LDL-C and HDL-C (38).

A study from Cameroon, West Africa among HIV patients who had initiated ART within six months documented elevated LDL-C and triglyceride levels but decreased HDL-C (31). While the studies from Cape Town, Uganda and a longitudinal study in Zimbabwe (42) compared lipid levels between ART- naïve patients and patients with a history of ART use, the other studies only focused on patients on ART.

However, a recent meta-analysis of African studies conducted by Dillon et al, (2) demonstrated that ART was associated with increased LDL and HDL levels (4)

Thus, the individual studies from Africa tend to show a positive effect of ART use on TC, LDL and triglyceride levels whereas there are some inconsistencies with regard to the effect of ART use on HDL levels.

Since most of these studies in Africa were hospital based and not at the population level, the introduction of biases is likely substantial. Most of the studies did not have HIV negative controls and did not consider a wide range of potential confounding factors.

Possible mechanisms: Cunha et al, (2015) described ART-associated dyslipidemia as the result of a complex of mechanisms involving aspects of immunological, hormonal, and genetic predisposition, as well as direct interactions between ARV metabolites (33). Studies have observed that ART impairs the hydrolysis of triglyceride-rich lipoproteins by tissue lipase resulting in a reduction in peripheral storage of free fatty acids (FFA).

The drugs also disrupt normal post-prandial catabolism of FFA (26). Protease inhibitors have been reported to inhibit degradation of apolipoprotein B resulting in increased levels in serum triglycerides, TC and LDL-C (43).

Combined PI and NRTI use inhibit preadipocyte maturation and therefore hasten peripheral lipoatrophy and cause a rise in triglycerides (43).

The FRAM study further supported this observation in that d4T and indinavir, a PI were reported to be associated with significant decreases in leg subcutaneous adipose tissue but not visceral adipose tissue (44).

1.2.6 Measuring lipids

A typical lipid profile (full lipogram) measures total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) (45). The TC is often used for screening purposes, assessing risk, monitoring treatment in cases of constraint resources and difficulty in obtaining LDL-C levels. The LDL-C levels however, are used for deciding on initiation of anti-lipid therapy (45, 46).

Lipid profile can be assessed using either serum in laboratory-based assays or via cost-effective ways such as point-of-care finger prick testing. For screening and initial diagnosis of dyslipidemia in the general population, current guidelines from South Africa recommend conducting a full lipogram (TC, HDL-C, LDL-C and TGs) using a finger prick testing (point-of care testing) device (46). Lipid profiles for this study were measured using point-of-care testing.

There are a number of different guidelines used for assessing dyslipidemia and these are mainly from Europe (47) and America (45) where populations are predominantly Caucasian.

Dyslipidemia for this study is defined using the South African dyslipidemia guidelines consensus statement, which was adopted from the European Society of Hypertension (ESH) and European Society of Cardiology guidelines (46).

The cut-offs used are a total cholesterol ≥ 6.21 mmol/L (112 mg/dL), and/or low-density lipoprotein cholesterol > 4.1 mmol/L (74 mg/dL), and/or high-density lipoprotein cholesterol < 1.19 mmol/L (21.42 mg/dL) and/or triglycerides > 2.25 mmol/L (40.5 mg/dL) (46).

1.2.7 Factors associated with dyslipidemia

Studies from high-income countries have documented that HIV infection and ART affect lipid levels (20). Many other factors modulate lipid levels and are often not measured in these studies so it is possible that the association between HIV and lipids is influenced by several other dependent covariates.

However, there is a paucity of data on factors associated with each of the serum lipid fractions – taking account of a wide range of confounding factors, including HIV infection and ART use in sub-Saharan Africa.

Some studies have measured a limited number of possible confounding factors in HIV positive subjects. Thus, Bekolo *et al* (2014) documented that viral load, current smoking, and > 2 years use of ART were positively correlated with LDL-C while alcohol was rather protective (31). A study in South Africa found a positive correlation between age, CD4 lymphocyte count, body mass index (BMI) and skin fold thickness, and triglyceride levels (32).

Studies from Nigeria observed that the WHO stages of HIV infection, and waist circumference, were positively correlated with all four molecules of serum lipids (28, 48). Studies in high-income countries demonstrated that men having sex with men (MSM), smoking, other metabolic diseases such as diabetes and regimens of ART were all associated with dyslipidemia (26).

Some studies have reported an association between moderate-to-vigorous physical activity and serum lipids (49, 50).

An earlier baseline study in the Agincourt population – from which participants were recruited for the current study – demonstrated that advanced age, sex, treatment history among HIV-positive patients and waist-to-hip ratio (WHR) were associated with lipid levels (51).

Furthermore, visceral adipose tissue (VAT) and subcutaneous adipose tissue (SCAT) have are predictors of serum lipid levels in another black South African population (52, 53).

However, no studies have investigated the effects of these body fat depots on lipid levels in HIV-positive subjects in Africa. A few studies conducted in the United States of America, have performed such investigations. These studies showed that decreased leg SCAT and more VAT among HIV positive men and women were associated with proatherogenic lipids levels (44, 54). The risk of dyslipidemia among HIV positive patients was likely mediated by HIV-associated lipoatrophy (54).

1.2.8 Cardiovascular risk

HIV/AIDS patients tend to have a higher prevalence of CVDs than the general population (2). This is primarily due to the high prevalence of pro-atherogenic lipid levels plus the prevailing traditional risk factors for CVDs that are also observed in the general population (51, 55).

Thus, studies among a cohort of HIV patients in the United Kingdom confirmed the increased risk of CVD in this population (5). Further studies among HIV patients in Brazil comparing the Framingham, PROCAM and DAD equations for assessing cardiovascular risk reported an increased risk among HIV patients than HIV negative controls (56).

Furthermore, a study applying the atherosclerotic cardiovascular disease (ASCVD) risk score on an HIV positive cohort in Gaborone, Botswana reported an increased risk of CVD among HIV positive patients compared to an HIV negative population (57). Other cross-sectional studies in West Africa found increased risk of CVD among HIV patients compared to HIV negative controls (58). These findings were confirmed by a cross-sectional study in a rural South African population (59).

Dyslipidemia causes increased cardiovascular risk in the general population as well. Longitudinal studies in the general population have reported increased hazards of cardiac events among people with dyslipidemia compared to those without (60).

Evaluation of cardiovascular risk among individuals without prior history of cardiovascular disease showed that high LDL-C and low HDL-C were significantly associated with later development of cardiovascular disease (61, 62).

1.3 Statement of the problem

Low and middle-income countries especially in sub-Saharan Africa have the highest global burden of HIV/AIDS with South Africa being the single country with the highest prevalence (9). There is also an evolving trend of an increasing burden of chronic diseases – including both non-communicable and infectious conditions – of which pro-atherogenic dyslipidemia among HIV infected patients is a major contributor (3).

Most of the studies on the association of HIV and ART with dyslipidaemia were conducted in high-income countries among Caucasians. In such countries, the HIV epidemic is mainly driven by special population groups such as men having sex with men, intravenous drug users, the increasing transgender population, and high influx of immigrants mainly from SSA (63).

The distribution of the HIV burden in sub-Saharan Africa, given an essentially heterosexual epidemic, is almost equal among males and females with a slight tilt towards females in the younger age groups. It is mainly driven by unprotected heterosexual intercourse, multiple sexual partners and concurrent sexually transmitted infection. Mother-to-child transmission (MTCT) resulting from inadequate access to treatment and high numbers of uncircumcised males are also major drivers of the epidemic in SSA. The voluntary medical male circumcision programme implemented in 14 priority countries (including South Africa) in the southern African region (63, 64) was meant to reduce the transmission of HIV.

Due to the relative contribution of these different population characteristics and prevailing confounding factors, findings from studies in high-income countries cannot be extrapolated to represent communities in low and middle-income countries, like those in sub-Saharan Africa.

Furthermore, large-scale population-based studies on the differential prevalence of dyslipidaemia among HIV patients compared to the general population are still lacking in sub-Saharan Africa. Also lacking, are studies that include the assessment of a broad array of factors that may modulate lipid levels including HIV-status and ART use.

This poses a constraint to the effective screening of CVD risk among PLWH. It has led to increasing calls to the research community to investigate this phenomenon.

1.4 Justification for the research

Current evidence suggests that many demographic, environmental and biological factors influence dyslipidemia. Failure to account for these is likely to result in a biased estimate of the association between HIV/ART use and dyslipidemia.

Longitudinal studies that effectively consider time give a clearer picture of this association however, population-based epidemiological studies – whether cross-sectional or longitudinal – considering a wide range of potential confounding factors can give a useful estimate of the association between lipid levels and effector variables.

Most of the studies in sub-Saharan Africa are hospital-based and have not considered a range of confounding factors which could affect the association between HIV/ART and serum lipid levels. This study examines a wide range of socio-demographic, environmental and anthropometric risk factors that could confound the association between HIV and ART on serum lipid levels.

Novel to this study is the use of visceral and subcutaneous adipose tissue measures as potential confounding factors. Most studies tend to use crude measures of central obesity such as waist and/or hip circumference. However, VAT and SCAT as ascertained using ultrasound scans offer greater precision to the measure of central obesity (65).

The study also draws its strength from the Agincourt health and socio-demographic surveillance system (HDSS) dataset, which involves a rich longitudinal cohort with in-depth assessment of many demographic and environmental factors that are pertinent to our study (66).

The study will therefore contribute to the growth of knowledge in this evolving field at a population level; and offer an assessment of the effect of VAT and SCAT on lipid levels in a population with a high prevalence of HIV-infection.

1.5 Main research question

What is the effect of HIV infection and ART use on serum lipid levels among adults in Agincourt, rural South Africa in 2015?

1.6 Study Aim

To determine the factors, including HIV infection and ART use, associated with lipid levels and dyslipidemia among adults in Agincourt, rural South Africa in 2015.

1.6.1 Study objectives

1. To describe the socio-demographic characteristics of adults aged 40+ years living in Agincourt, rural South Africa in 2015.

2. To compare lipid levels and prevalence of dyslipidemia between HIV-negative, HIV-positive but ART-naive and HIV-positive but ART-exposed adults in Agincourt, South Africa in 2015.

3**.** To identify the factors associated with the four lipid species and dyslipidemia among adults in Agincourt, South Africa in 2015.

2.0 CHAPTER 2: MATERIALS AND METHODS

This chapter describes the materials and methods used in the primary study. Brief descriptions to the study setting, study design and participant characteristics are in this chapter. The chapter further describes the data management process and statistical analysis plan employed to arrive at the results of this nested study.

2.1 PRIMARY STUDY

2.1.1 Background of primary study

This study is nested in two collaborative studies within the MRC/Wits Rural Public Health and Health Transitions Research Unit based in the Agincourt sub-district:

- The Africa Wits-INDEPTH partnership for Genomic studies on cardiometabolic diseases (AWI-Gen) of the Human Heredity and Health in Africa (H3A) Consortium is a partnership between the University of the Witwatersrand and the International Network for the Demographic Evaluation of Populations and their Health in low and middle-income countries (INDEPTH) (67).
- The Health and Aging in Africa: Longitudinal Studies of an INDEPTH Community (HAALSI), in Agincourt is a partnership between Harvard University, USA, University of the Witwatersrand and the INDEPTH Network (68).

The HAALSI/AWI-Gen collaboration aims to study the interaction of communicable and non-communicable diseases and their potential impact on adult health and ageing (69). The AWI-Gen study (40-60 years) was a subset of the HAALSI study that included participants of 40+ years. The AWI-Gen study in addition included extensive anthropometric measurements while the HAALSI study included extensive measurements on cognition and ageing.

2.1.2 Study setting

The HAALSI/AWI-Gen collaborative studies were conducted in the MRC/Wits Rural Public Health and Health Transitions Research Unit in Agincourt sub-district, Mpumalanga province. The Agincourt Health and socio-Demographic Surveillance Site (HDSS) located in northeast South Africa and bordered by Mozambique is one of the founding members of the INDEPTH network (66).

It was established in 1992 and has since been monitoring migrations and health events in an area that covers a land mass today of 450km^2. The population of the area has grown from 57 600 people in 8,900 households in 20 villages to 90,036 people living in 14,382 households in 27 villages by the end of 2012(66). The major increase in the population is mainly due to the influx of Mozambican immigrants into the area in the wake of the civil war in Mozambique.

While the study setting has a relatively good representation of educational institutions, the quality is rather low with relatively few students progressing past secondary school level. Two health centres and six clinics provide public health care services in the study area (66).

The sub-district has an average HIV prevalence of 19.4% (6). The average prevalence of abdominal/central obesity is 46.3% among men and 90.3% among women (51) and HIV and TB are the main causes of adult related mortalities – although mortality from non-communicable causes is increasing.

2.1.3 Study design

Both studies were population-based baseline / cross-sectional studies based on the longitudinal cohort of adults monitored by the Agincourt HDSS.

2.1.4 Study population

The AWI-Gen study consisted of approximately 2500 adults aged 40-60 years while the overlapping HAALSI study consisted of approximately 5 000 adults aged 40+ years all of whom lived in the Agincourt sub-district from 2013 to 2016 when the data collection was carried out. Both studies have a major focus on cardiometabolic disease and the biological, behavioural and environmental determinants and consequences.

2.1.5 Sampling

Both studies used random sampling from the HDSS database to select participants from households under surveillance in the study area. However, samples stratified to reflect an equal number of men and women by age group.

2.1.6 Data collection

All participants provided written informed consent before any study procedures were performed.

Both studies used interview-administered questionnaires to collect information on participants. Trained local research assistants administered these questionnaires.

In addition to socio-demographic characteristics collected in common by both studies, the AWI-Gen study collected information on visceral and subcutaneous adipose tissue, history of co-morbidities e.g. diabetes, self-reported ART status, dietary history, physical activity and genetic data. The HAALSI study, in addition, collected information on mental state, cognition, laboratory assessment of HIV status and point-of-care measurements of lipid and glucose levels. Point of care lipids and questionnaire were measured in the field while participants were invited to the study site for further anthropometric measures on a different visit day.

2.1.7 How key variables from the primary study were measured

2.1.7.1 Height and Weight

With participants in light clothing and without shoes, height was measured using a height sensor with infrared measurement and weight was measured using Genesis Growth Management Scale from Patient Focus Africa. A trained research officer carried out both measurements.

2.1.7.2 Waist and Hip circumference

The waist circumference of the participant were measured to the nearest 0.5cm between the lower margin of the least palpable rib and the iliac crest using a stretch-resistant tape measure (SECA) according to the guidelines of the WHO 2008 report on waist circumference and waist-hip ratio (70). The hip circumferences were measured from the outermost part of the gluteus.

Body mass index (BMI) and waist-to-hip ratio (WHR) were then derived from these measurements using WHO (71) recommended cut-offs.

2.1.7.3 Visceral and Subcutaneous adipose tissue (VAT and SCAT)

A LOGIQ e ultrasound system (GE Healthcare, CT, USA) with a 2-5 MHz 3C-RS curved array transducer was used to determine VAT and SCAT thicknesses. USS VAT thickness was defined as the distance (cm) from the peritoneum to the vertebral bodies, and USS SCAT thickness was defined as the depth (cm) from the skin to the linea alba.

The scan depth was set at 15 cm for the visceral fat measure and 9 cm for the subcutaneous fat measure to visualize the relevant anatomical structures. Both measurements were obtained where the xyphoid line and the waist circumference met (53, 65). The coefficient of variation for USS measurement for the HAALSI/AWI-Gen collaborative studies ranged from 5-8%.

2.1.7.4 Moderate-to-vigorous physical activity (MVPA)

The global physical activity questionnaire (GPAQ) was used to assess self-reported physical activity (72). The total moderate-vigorous physical activity (MVPA) in minutes per week (mins/wk) was calculated from the accumulated occupation, travel-related and leisure time physical activity.

2.1.7.5 Diabetes status

Diabetes was defined from self-report of ever being diagnosed with diabetes or glucose ≥ 7 mmol/l in fasting group (defined as > 8 hours), or glucose ≥11.1 mmol/l in non-fasting ("random or casual") group (73). Individuals with missing fasting information were considered to be not fasting.

2.1.7.6 HIV status

For prevalence of HIV, we used the results from blood using a protocol of sequential assays. Specifically, the Vironostika Uniform 11 (Biomeriuex, France) screening assay was used to detect the presence of the virus and in samples that tested positive the Roche Elecys (USA) assay (confirmatory) was used to determine the viral load on DBS.

Contradictory results were resolved using the Siemens Centaur XP (USA) immunoassay, in accordance with World Health Organization (WHO) guidelines (74, 75). All individuals who consented to the blood collection and testing regardless of prior knowledge of their status were subsequently tested.

2.1.7.7 ART status

Use of ART assessed by self-report. Results from laboratory confirmation were not immediately available at the time of writing this report.

2.1.7.8 Lipid profile

Lipid profiles (non-fasting) were measured using a calibrated point-of-care (POC) device**, CardioChek® PA silver version** by Polymer Technology Systems, Inc. Indianapolis, Indiana, USA.

2.2 SECONDARY DATA ANALYSIS METHODS

2.2.1 Study design

This secondary analysis is a population based cross-sectional study nested within the two collaborative studies described above.

2.2.2 Data source

Both the scientific leadership of the MRC/Wits Rural Public Health and Transitions Research Unit and the Sydney Brenner Institute of Molecular Bioscience, University of the Witwatersrand, Johannesburg, South Africa, provided data for this secondary analysis.

2.2.3 Study population

Adults age 40+ years living in the Agincourt sub-district HDSS coverage area from 2013 to 2016.

2.2.4 Study sample

We merged the two datasets from AWI-Gen and HAALSI to produce a sub-sample of 2 344 adults aged 40+ years. We then included all participants with data on exposure and outcome of interest into this secondary analysis and 2110 individual met this inclusion criterion and was thus used for the final analysis. A retrospective power calculation using a two-sample proportions test used to determine the adequacy of this sample.

The proportion of HIV negative (75.6%) and HIV positive (24.2%) participants with dyslipidemia were used for the power calculation. With hypothesis that HIV and ART causes an increase in ones risk of dyslipidemia, we sought to determine the power that would detect a difference in proportions at an alpha level of 5%.

The estimated power to detect an absolute increase of 10% in prevalence of dyslipidemia for our sample was almost 100%. We concluded this sample adequately powered to answer our research question.

2.2.5 Selected variables for the secondary analysis

2.2.5.1 Independent/Exposure variable

The exposure variables were the HIV status provided by the HAALSI study and ART status as provided by the AWI-Gen and HAALSI studies.

2.2.5.2 Dependent/Outcome variable

The outcome variables were each molecule of the serum lipid profile: total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C)

2.2.5.3 Covariates/Predictor variables

These selected from variables measured in the combined studies and their selection for use in the multivariable regression models and guided by prior knowledge taken from the current literature. These included socio-demographic, behavioural and dietary variables, and clinical and anthropometric variables, as described below.

2.2.5.4 Socio-demographic variables

Data collected for age, sex, and marital status, highest level of education, employment status, household wealth asset index and household size (number of individuals living in the household). Participants' ages were categorized into age groups (40-44; 45-54; 55-64; 65+) according to the South African National Health and Nutrition Examination Survey categorization (SANHANES) (76).

2.2.5.5 Behavioural physical activity and dietary variables

These included alcohol use, smoking history, and average sleep in hours per day and moderate-to-vigorous physical activity (MVPA). Dietary variables included number of servings of fruits and vegetables per day and number of sugar-sweetened beverages (SSB) consumed per day.

2.2.5.6 Clinical and anthropometric variables

The main clinical variable measured was diabetes status as a co-morbidity that can influence lipid levels.

Anthropometric variables included weight, height, waist and hip circumference, visceral adipose tissue and subcutaneous adipose tissue. Weight and height were used to generated body mass index (weight [kg]/height^2 [m]). BMI was then categorized according to the WHO Expert Committee report on physical status into: underweight: <18.5, Normal: 18.5 to < 25, Overweight 25 to <30, Obese: >= 30 (71). The VAT and SCAT data provided by the AWI-Gen study.

2.3 Data management and analysis

2.3.1 Data management

Relevant variables for these participants extracted and checked for completeness, consistency and duplicates. Frequency tables used to check for missing data. Two of the continuous variables had more than 20% missing data: subcutaneous adipose tissue, 24.7%; and visceral adipose tissue, 24.8%.

In order to ensure precision and eliminate bias, mean value imputations done for missing data. The following variables were selected, based on prior knowledge, as strong correlates of the missing variables; age, sex, HIV status, BMI and diabetes. These variables used to replace each missing value with the mean of the observed values for that variable. The drawback to this is underestimation of the standard deviation and distortion of the estimated association towards zero (77).

We subsequently built our models with the original variables and later on with the imputed variables. We then compared the changes in the R-squared value, the direction of association and

level of significance. Where the magnitude of change in effect measure (or R-squared of the model) and level of significance was not significantly different (<10% change), we maintained the model that did not include the imputed data. Where a significant difference was observed, we maintained the imputed data in the model.

The final cleaned dataset containing 2110 participants is used for all data analysis in this study. Relevant variables were then calculated e.g. BMI, WHR, high TC, high TGs, low HDL-C, high LDL-C and dyslipidemia.

2.3.2 Generating and re-categorization of key variables

2.3.2.1 Exposure status

A combined exposure status was generated based on HIV and ART status. Three mutually exclusive categories; HIV negative, HIV positive plus ART use and HIV positive but ART naive were generated.

2.3.2.2 Dyslipidemia

Dyslipidemia was diagnosed if one or more of the following criteria were met: elevated total cholesterol (≥6.21 mmol/L), low HDL (1.19 mmol/L), elevated LDL (>4.1 mmol/L), elevated triglycerides (>2.25 mmol/L) (46) and if the subject reported being clinically diagnosed with and receiving treatment for high cholesterol. A binary outcome generated using above criteria to describe dyslipidemia as "Yes" or "No".

2.3.3 Statistical analysis

Both descriptive and inferential statistical analyses conducted in this study. STATA version 13 (StataCorp LP, Texas USA) was used to perform all the analyses.

2.3.2.1 Descriptive analysis

Frequencies were generated using cross-tabulation to describe the categorical characteristics of study participants stratified according to sex. Continuous variables are expressed as median (inter-quartile range) if skewed and mean ± SD for normally distributed data. Pearson's Chi

square (χ^2) or Fisher's exact test used to compare differences in categorical variables between groups whilst the t-test used to compare differences in normally distributed continuous variables.

A non-parametric test, the Wilcoxon–Mann–Whitney U two-sample test (Rank sum test) used for comparing gender differences for skewed continuous data. A $p < 0.05$ considered statistically significant. One way ANOVA used to compare differences in mean serum lipid levels across the three exposures categories (HIV negative, HIV+ ART+ and HIV+ ART naive).

2.3.2.2 Multivariable linear regression analyses

Multivariable linear regression models were built for each of the 4 lipid species.

The outcome variables were normally distributed hence there was no need for transformation to normality. In building the linear models, we examined the independent association between the exposure (HIV-, HIV+ on ART and HIV+ ART naive) and each of the outcome (LDL-C, HDL-C, TC and TGs) variables and the relationship of each of the selected predictor variables with each of the outcome variables in univariable linear regression analyses.

A stepwise forward selection method used in building the models. Variables that correlated with lipids at $p \leq 0.2$ in the univariable linear regression analysis were included in the multivariable linear regression analysis. We also checked for multicollinearity between related variables particularly the anthropometric variables using the variance inflation factor (VIF). Variables with a VIF greater than 10 dropped from the model.

Unstandardized beta coefficients with 95% confidence intervals (95% CI) were used to measure the magnitude of association and $p < 0.05$ was considered statistically significant for the final model. For categorical variables with more than two levels, a post estimation test ("testparm") was used to examine the overall significance of the said variable in the model and this generates a single p-value.

We used magnitude of R-squared to compare the various models generated, aiming at a higher R-squared for the final model. This is because the R-squared gives an idea of the proportion of the variance of the dependent variable that is explained by the model.

Additionally, Akaike's and Schwarz's Bayesian information criteria (AIC and BIC) post-estimation test was used to compare the fitted models. The final model had the lowest AIC and BIC.

The Breusch-Pagan/Cook-Weisberg test for homogeneity of variance was used to ensure residuals of each of the final models were approximately normally distributed / homoscedastic.

The final parsimonious model from the linear regression analysis was checked for model specifications and for omitted-variables bias using the Ramsey regression specification-error test.

2.3.2.3 Multivariable logistic regression analyses

Each of the four lipid fractions were re-categorized into binary outcomes i.e. Increased TC, increased TGs, increased LDL-C and decreased HDL-C. The independent association of HIV/ART status with each of these four binary outcomes was determined using univariable logistic regression.

A final binary outcome variable, dyslipidemia was generated by combining each of the four lipid molecule categorical variables, as described previously using standard guidelines.

We initially determined the independent association of each of the selected covariates or predictor variables with dyslipidemia in a univariable logistic regression analysis. Variables significant at a $p<0.2$ were then included in a multivariable logistic regression analysis. A variance-covariance (robust estimation) approach was used to estimate standard errors in the models.

Multicollinearity was determined by ensuring that the VIF for the variables in the final model was less than 10. The Wald test was used to compare significance between the various models generated and the final parsimonious model had a higher Wald test.

As explained for the linear regression models, the categorical variables with more than two levels were analysed using a post estimation test to determine the overall significance of the said variable in the model and this produced a single p-value.

Odds ratios (OR) with 95% confidence interval (95% CI) were used to measure the magnitude of association and $p < 0.05$ was considered statistically significant. The final parsimonious model from the logistic regression analysis checked for model specifications goodness of fit test.

2.4 Ethical consideration

The main AWI-Gen/HAALSI collaborative studies at Agincourt received ethical clearance from the University of the Witwatersrand Human Research Ethics Committee (ethics approval numbers: M121029 and M110138, respectively). Participants at recruitment signed written informed consent before all study procedures.

Further ethical approval for this secondary analysis, was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg. Ethics certificate number: M161197.

3.0 CHAPTER 3: RESULTS

This chapter provides detailed results for this study.

3.1 Socio-demographic characteristics of study participants

Results of the secondary analysis of 2 110 participants are presented.

Table 3.1a shows the socio-demographic characteristics of the study participants stratified by sex.

The participants consisted of 838 (39.7%) men and 1 272 (60.3%) women and the mean (± SD) age was 58.54 ± 10.91. There was a significant difference in mean (± SD) age among women (59.86 ± 11.03) and men (57.67 ± 10.7 years; $p < 0.0001$).

The largest groups of the study participants (31.8%) were in the 65+ year's group with more men (36.2%) than women (29.0%) in that age group ($p < 0.0001$). While the 40-44-year-old group contributed a smaller number of participants with 11.3% males and 12.5% females.

Over 50% of the participants had some form of formal education from the primary level to tertiary level. Of those that had, no form of formal education there were more women (43.8%) compared to men (35.8%; $p < 0.0001$). There was a moderately high employment rate of 72.7%, with slightly more men (73.1%) employed compared to women (72.5%) (See Table 3.1a).

More men were currently married (68.9%) than women (42.2%) who were more likely to be widowed (39.6%) when compared to men (10.4%). These demographic differences were highly significant ($p < 0.0001$).

There was no difference between men and women when it came to household wealth asset index, ($p = 0.579$). A majority of the participants lived in a household composed of seven or more people. We observed that fewer women (6.4%) were likely to be living alone compared to men (14.0%; $p < 0.0001$) (Table 3.1a).

Table 3.1a Socio-demographic characteristics of adults in Agincourt sub-district in 2015

	Women (n=1272, 60.3%)	**Men (n=838, 37.7%)**	**Total (N=2110)**	
	n (%)	**n (%)**	**N (%)**	***p*-value**
Age in years -mean ± SD	59.86 ± 11.03	57.67 ± 10.7	58.54 ± 10.91	< 0.0001
Age groups in years [a]				
40-44	168 (13.2)	95 (11.3)	263 (12.5)	< 0.0001
45-54	394 (31.0)	199 (23.7)	593 (28.1)	
55-64	341 (26.8)	241 (28.8)	582 (27.6)	
65+	369 (29.0)	303 (36.2)	672 (31.8)	
Country of origin				
South Africa	871 (68.5)	614 (73.3)	1 485 (70.4)	0.020
Mozambique/Other	400 (31.5)	224 (26.7)	624 (29.6)	
Education category				
No formal education	556 (43.8)	299 (35.8)	855 (40.6)	< 0.0001
Some primary (1-7 years)	421 (33.1)	351 (42.0)	772 (36.7)	
Some secondary (8-11 years)	137 (10.8)	104 (12.4)	241(11.4)	
Secondary or more (12+ years)	156 (12.3)	82 (9.8)	238 (11.3)	
Marital status				
Never married	60 (4.7)	55 (6.6)	115 (5.5)	< 0.0001
Separated / divorced	171 (13.4)	119 (14.2)	290 (13.7)	
Widowed	504 (39.6)	87 (10.4)	591 (28.0)	
Currently married	537 (42.2)	577 (68.9)	1 114 (52.8)	
Employment status				
Employed (part or full time)	922 (72.5)	610 (73.1)	1 532 (72.7)	0.002
Not working	171 (13.5)	144 (17.2)	315 (15.0)	
Homemaker	178 (14.0)	81 (9.7)	259 (12.3)	
Household size [b]				
Living alone	81 (6.4)	117 (14.0)	198 (9.4)	< 0.0001
Living with one other person	122 (9.6)	81 (9.7)	203 (9.6)	
Living in 3-6 person household	643 (50.6)	370 (44.2)	1 013 (48.0)	
Living in 7+ person household	426 (33.5)	270 (32.2)	696 (33.0)	
Wealth asset index [c]				
1(Poorest)	262 (20.6)	183 (21.8)	445 (21.1)	0.579
2	254 (20.0)	169 (20.2)	423 (20.0)	
3	245 (19.3)	160 (19.1)	405 (19.2)	
4	261 (20.5)	149 (17.8)	410 (19.4)	
5 (Wealthiest)	250 (19.7)	177 (21.1)	427 (20.2)	

[a]Age groups based on SANHANES and DHS surveys; [b]Household size: Categorical number of individuals living in a household; [c] Based on household asset index score in 2009.

There were a high proportion of migrants from Mozambique (29.6%) in Agincourt.

3.2 Behavioural characteristics of study participants

In Table 3.1b, we summarized results for behavioural, dietary and physical activity of study participants.

Men and women differed significantly by alcohol consumption and smoking history. Thus, 70.4% of men consumed alcohol compared to 24% of women ($p < 0.0001$). Close to half, (48%) of the men had either previously smoked or were current smokers compared to less than 2% of women (1.7%; $p < 0.0001$).

Table 3.1b Behavioural, dietary and physical activity profile of adults in Agincourt in 2015

	Women (n=1272, 60.3%)	**Men (n=838, 37.7%)**	**Total (N=2110)**	***p*-value**
Alcohol consumption - n (%)				
Never	966 (75.9)	248 (29.6)	1 214 (57.5)	< 0.0001
Ever and current	306 (24.1)	590 (70.4)	896 (42.5)	
Smoking history - n (%)				
Never	1 250 (98.3)	433 (51.7)	1 683 (79.8)	< 0.0001
Ever and current	22 (1.7)	405 (48.3)	427 (20.2)	
Physical activity - median (IQR)				
Average sleep time (hours per night)	8.86 (5.00-13.71)	9.00 (5.00-14.00)	9.00 (5.00-14.00)	0.575
Moderate-to-Vigorous physical activity (MVPA) (minutes/week)	300 (0-1440)	420 (0-1440)	360 (0-1440)	0.008
Dietary history - median (IQR)				
Fruit intake (servings per day)	1 (1-3)	1.5 (1-2)	1 (1-5)	0.729
Vegetable intake (servings per day)	2 (1-14)	2 (1-3)	2 (1-14)	0.865
Sugar Sweetened Beverages, SSB (number/week)	1 (0-6)	0.5 (0-2)	1(0-6)	0.888

There was no difference in dietary patterns (servings of fruits; $p = 0.729$ and vegetables; $p = 0.865$ and number of sugar sweets and beverages per week; $p = 0.888$) between men and women.

The median (IQR) average sleep time per day was 9 hours (5-14) and there was no difference between men and women ($p = 0.575$). The median (IQR) moderate-to-vigorous physical activity for the study population was 360 (0-1440) minutes per week, and was significantly higher in men than women ($p = 0.008$).

3.3 Clinical history and anthropometric measurements of study participants

Diabetes was the metabolic disorder measured in this study as a clinical co-morbidity that can influence serum lipids. There was no significant difference in diabetes status between men and women ($p = 0.804$) (Table 3.1c).

Men and women differed significantly for all the anthropometric measurements obtained in this study (Table 3.1c).

The mean (± SD) waist circumference was 96.14 ± 15.70 cm for women and 89.54 ± 13.83 cm for men ($p < 0.0001$). The hip circumference was 107.58 ± 14.78 cm for women and 96.75 ± 11.62 cm for men ($p < 0.0001$). The mean (± SD) BMI in kg/m^2 for men was 24.73 ± 5.68 and for women was 29.74 ± 6.65, and this was statistically different ($p < 0.0001$). Men had a higher mean (± SD) waist-to-hip ratio compared to women ($p < 0.0001$). Obesity (BMI > 30 kg/m^2) was more common among women than men ($p < 0.0001$).

This however was not the same for central obesity using ultrasound measurements of visceral and subcutaneous adipose tissue. The mean VAT did not differ between men (5.39 ± 2.87 cm) and women (5.09 ± 2.65 cm; $p < 0.118$), whilst the mean SCAT was higher in women (3.21 ± 2.21 cm) than in men (2.33 ± 2.46 cm; $p < 0.0001$) (Table 3.1c).

Table 3.1c Clinical and anthropometric characteristics of adults in Agincourt in 2015

	Women (n=1272, 60.3%)	**Men (n=838, 37.7%)**	**Total (N=2110)**	***p*-value**
Clinical history				
Diabetes - n (%) [e]	142 (11.3)	97 (11.7)	239 (11.5)	0.804
Waist and hip measurements				
Waist circumference (cm) - mean ± SD	96.14 ± 15.70	89.54 ± 13.83	93.53 ± 15.33	<0.0001
Hip circumference (cm) - mean ± SD	107.58 ± 14.78	96.75 ± 11.62	103.39 ± 14.68	<0.0001
Waist-to-hip ratio - mean ± SD	0.89 ± 0.08	0.92 ± 0.07	0.91 ± 0.08	<0.0001
Body mass index (BMI)				
BMI (kg/m^2) - mean ± SD	29.74 ± 6.65	24.73 ± 5.68	27.76 ± 6.74	<0.0001
BMI (kg/m^2) category - n (%) [d]				
Underweight	20 (1.6)	80 (9.8)	100 (4.8)	<0.0001
Normal	316 (25.1)	396 (48.3)	712 (34.2)	
Overweight	367 (29.1)	215 (26.2)	582 (28.0)	
Obese	558 (44.3)	129 (15.7)	687 (33.0)	
Ultrasound measures of central obesity				
Visceral adipose tissue (cm) - mean ± SD	5.09 ± 2.65	5.39 ± 2.87	5.22 ± 2.76	0.118
Subcutaneous adipose tissue (cm) - mean ± SD	3.21 ± 2.21	2.33 ± 2.46	2.82 ± 2.37	<0.0001
VAT:SCAT - mean ± SD	2.59 ± 1.90	5.26 ± 3.94	3.78 ± 3.26	<0.0001

[d] BMI category (Underweight: <18.5, Normal: 18.5 to < 25, Overweight: 25 to <30, Obese: ≥ 30) according to WHO (71); [e]Diabetes definition: Self reported diabetes diagnosis OR glucose ≥ 7 mmol/l (126 mg/dL) in fasting group (defined as > 8 hours), glucose ≥11.1 mmol/l (200 mg/dL) in non-fasting ("random or casual") samples (73). Individuals with missing fasting information considered to be not fasting.

3.4 Participant characteristics by exposure and outcome (lipids) status stratified according to sex

The HIV prevalence in this population was 15.7%. There was no difference (p = 0.319) in prevalence between women (16.4%) and men (14.7%) (see Table 3.2a).

Table 3.2a HIV/ART exposure status of men and women in Agincourt in 2015

HIV/ART status	Women n (%)	Men n (%)	Total N (%)	P-value
HIV negative	922 (83.6)	634 (85.3)	1 556 (84.3)	0.319
HIV+ (Combined)	181 (16.4)	109 (14.7)	290 (15.7)	

Concerning lipid measurements, nearly half (43.7%) of the participants had dyslipidemia with no observed differences between women and men (p = 0.627; see Table 3.2b).

Table 3.2b Serum lipid levels of men and women in Agincourt in 2015

Serum lipids	Women mean ± SD	Men mean ± SD	Total mean ± SD	P-value
Total Cholesterol (mmol/L)	4.59 ± 1.37	3.76 ± 0.96	4.32 ± 1.30	0.082
Triglycerides (mmol/L)	1.84 ± 1.17	1.61 ± 0.79	1.77 ± 1.06	0.554
HDL-C (mmol/L)	1.69 ± 0.65	1.89 ± 0.46	1.76 ± 0.59	0.364
LDL-C (mmol/L)	2.24 ± 1.16	1.26 ± 0.68	1.89 ± 1.11	0.023
Dyslipidemia - n (%) [g]	524 (44.2)	329 (43.1)	853 (43.7)	0.627

[g]Dyslipidemia broad definition: Elevated TC (≥6.21 mmol/L), OR Elevated triglycerides (>2.25 mmol/L), OR Low HDL (<1.19 mmol/L), OR Elevated LDL (>4.1 mmol/L), OR reports ever diagnosed with high cholesterol (46).

However, when looking at individual lipid types, the mean (±SD) total cholesterol for adults in Agincourt was 4.32 ± 1.30 mmol/L with marginal differences between men and women (p=0.082) (Table 2b). Mean triglyceride (p = 0.554) and HDL-C (0.364) levels did not differ between men and women. The mean (±SD) LDL-C for women was 2.24 ± 1.16 mmol/L and that for men was 1.89 ± 1.11 mmol/L which was statistically different (p = 0.023).

3.5 Lipid levels and prevalence of dyslipidemia according to HIV and ART status

Prevalence and 95% confidence (CI) of dyslipidemia and abnormal levels of each of the four molecules of serum lipids by exposure status (HIV/ART status) are present in Table 3.3 below.

Table 3.3 Levels and prevalence of dyslipidemia with 95%CI by exposure status of adults in Agincourt in 2015

Lipid levels	**HIV negative Mean ± SD**	**HIV positive on ART Mean ± SD**	**HIV positive ART naïve Mean ± SD**	**Total (N = 1712) [h] Mean ± SD**	***p*-value [i]**
Total Cholesterol	4.23 ± 1.24	4.14 ± 1.12	3.96 ± 1.09	4.20 ± 1.22	0.091
Triglycerides	1.76 ± 0.95	1.69 ± 0.79	1.75 ± 0.96	1.75 ± 0.94	0.002
HDL-C	1.54 ± 0.49	1.71 ± 0.53	1.39 ± 0.49	1.56 ± 0.51	0.453
LDL-C	2.12 ± 0.99	1.96 ± 0.97	1.83 ± 0.97	2.08 ± 0.98	0.910
Prevalence of dyslipidemia (95% CI)					***p*-value**
Dyslipidemia [j]	44.5 [42.0, 47.1]	35.8 [30.0, 42.0]	64.7 [40.4, 83.2]	43.5 [41.1, 45.8]	0.008
High total cholesterol [k]	5.8 [4.7, 7.2]	4.1 [2.6, 7.8]	-	5.5 [4.5, 6.7]	0.324
High triglycerides [l]	22.1 [20.0, 24.3]	17.2 [13.0, 22.5]	31.3 [13.6, 56.7]	21.5 [19.6, 23.5]	0.145
High LDL [m]	3.5 [2.6, 4.7]	2.7 [1.2, 5.9]	6.7 [3.5, 9.0]	3.4 [2.6, 4.5]	0.013
Low HDL [n]	26.5 [24.3, 28.9]	20.0 [15.5, 25.5]	47.1 [27.5, 69.8]	25.8[23.8, 27.9]	0.652

[h] 288 participants were excluded from prevalence analysis because they did not have results for all four types of the serum lipids.
[i] One way ANOVA was used to compare differences in mean serum lipid levels across exposure status.
[j] Dyslipidemia definition: Elevated TC (≥6.21 mmol/L), AND/OR Elevated triglycerides (>2.25 mmol/L), AND/OR Low HDL (<1.19 mmol/L), AND/OR Elevated LDL (>4.1 mmol/L), OR reports of ever diagnosed with high cholesterol.
[k]Elevated total cholesterol (≥6.21 mmol/L); [l]Elevated triglycerides (>2.25 mmol/L); [m]Low HDL (<1.19 mmol/L); [n] Elevated LDL (>4.1 mmol/L) (46).

The prevalence of dyslipidemia in the entire population was 43.5% (95% CIs, 41.1, and 45.8) (see Table 3.3). Dyslipidemia differed by exposure status (p=0.008) (see Table 3). It was highest among HIV+ participants who were ART naïve (64.7% [40.4, 83.2]). There was no difference

for high total cholesterol (p=0.324), high triglycerides (p=0.145) and low HDL-C (p=0.652) (see Table 3) across the exposure status.

However high LDL-C differed across the three exposure groups (p=0.013). Thus, HIV+ participants who were ART naïve had the highest prevalence of high LDL-C (6.7% [3.5, 9.0]) followed by the HIV negative participants (3.5% [2.6, 4.7]) and HIV + participants on ART had the lowest prevalence (2.9% [1.2, 5.9]).

3.6 Factors associated with serum lipids

We initially determined the association of each independent variable with each of the four species of lipids. We then included all variables with $p < 0.2$ in the univariable analysis in a multivariable linear regression model and the outcomes are shown in Tables 3.4 to 3.7.

Factors significant at the multivariable analysis are bolded in the tables with their respective p-values. Age, sex and HIV/ART status irrespective of the p-value in the univariable analysis were included in multivariable analysis. Due to the relatively small numbers of person with HIV+ but ART naïve (n=18), a sensitivity analysis was done using the adjusted regression models with this group and with this group combined with the HIV+ ART+ group (see Appendix 3). The results were similar hence; we present the model with the HIV+ART naive group. However, these results should be interpreted with caution due to the small number of subjects in this group.

3.6.1 Factors associated with total cholesterol (TC)

At the multivariable level, there were significant associations between the following factors and TC: age ($p = 0.014$), sex ($p = 0.019$), diabetes ($p = 0.039$) alcohol consumption ($p = 0.038$) and BMI ($p = 0.030$) (see Table 3.4). A unit increase in participant's age was associated with a 0.01 mmol/L increase in TC, whilst males had a 0.52 mmol/L lower level of TC compared to females. Though marginally significant ($p = 0.093$), a higher level of education was associated with a reduction in TC levels (see Table 3.4).

Table 3.4 Factors associated with total cholesterol in Agincourt, rural South Africa, 2015

Covariates	Univariable analysis		Multivariable analysis	
	β-coefficients (95%CI)	***p*-value**	**β-coefficients (95%CI)**	***p*-value**
Age	0.0146 (0.011, 0.021)	< 0.0001	**0.024 (0.010, 0.031)**	**0.014**
Sex				
Female	Ref	Ref	Ref	Ref
Male	-0.372 (-0.491, -0.273)	< 0.0001	**-0.315 (-0.572, -0.051)**	**0.019**
Exposure status				
HIV negative	Ref	Ref	Ref	Ref
HIV+ ART use	-0.092 (-0.265, 0.071)	0.372	0.014 (-0.264, 0.279)	0.684
HIV+ ART naive	-0.264 (-0.851, 0.324)		0.412 (-0.510, 1.329)	
Education Status				
No formal education	Ref	Ref	Ref	Ref
Some primary (1-7 years)	-0.133 (-0.266, -0.009)	0.0007	-0.081 (-0.291, 0.135)	0.093
Some secondary (8-11 years)	-0.290 (-0.482, -0.119)		-0.230 (-0.541, 0.074)	
Secondary or more (12+ years)	-0.291 (-0.484, -0.115)		-0.394 (-0.720, 0.075)	
Marital Status				
Currently married	Ref	Ref	Ref	Ref
Never married	0.031 (-0.280, 0.213)	0.0054	0.304 (-0.092, 0.691)	0.289
Separated/Divorced	0.084 (-0.089, 0.251)		0.195 (-0.080, 0.461)	
Widowed	0.221 (0.091, 0.354)		0.016 (-0.235, 0.255)	
Diabetes mellitus				
No	Ref	Ref	Ref	Ref
Yes	0.173 (-0.021, 0.356)	0.054	**0.310 (0.019, 0.614)**	**0.039**
Alcohol consumption				
Never	Ref	Ref	Ref	Ref
Ever or current	-0.150 (-0.262, -0.041)	0.009	**0.250 (0.021,** 0.480**)**	**0.038**
Smoking History				
Never	Ref	Ref	Ref	Ref
Ever or current	-0.344 (-0.481, -0.203)	< 0.0001	-0.235 (0.524, 0.055)	0.102
Body mass index	0.029 (0.023, 0.031)	< 0.0001	**0.024 (0.018, 0.041)**	**0.030**
Hip circumference	0.014 (0.010, 0.025)	< 0.0001	-0.013 (-0.021, 0.015)	0.160
VAT:SCAT ratio	-0.032 (-0.061, -0.010)	0.011	-0.013 (-0.041, 0.022)	0.649

3.6.2 Factors associated with triglycerides (TGs)

Table 5 shows results of univariable and multivariable linear regression analyses used to determine factors associated with serum TGs among participants.

Factors associated with serum TGs at the univariable level at $p < 0.2$ are summarized in Table 3.5 below. The HIV status was not independently associated with serum TGs ($p = 0.553$).

The following were significantly associated with serum TG levels in the multivariable model: age (p = 0.003), BMI (p = 0.044), diabetes mellitus (p < 0.0001), waist circumference (p < 0.0001) and hip circumference (p = 0.001) (see Table 3.5). Except for male gender and hip circumference, the other factors were positively correlated with TGs with diabetes causing the highest increase in serum TGs by 0.27mmol/L (0.27 [0.13, 0.40]; p < 0.0001) (see Table 3.5).

Table 3.5 Factors associated with triglycerides levels among adults in Agincourt, 2015 (from a linear regression analysis)

Covariates	**Univariable analysis**		**Multivariable analysis**	
	β-coefficients (95%CI)	**P-value**	**β-coefficients (95%CI)**	***p*-value**
Age	0.012 (0.010, 0.035)	0.002	**0.013 (0.011, 0.035)**	**0.003**
Sex				
Female	Ref	Ref	Ref	Ref
Male	-0.120 (-0.219, -0.041)	0.004	**-0.099 (-0.198, 0.010)**	**0.053**
Exposure status				
HIV-	Ref	Ref	Ref	Ref
HIV+ ART use	-0.071 (-0.190, 0.062)	0.553	0.065 (-0.060, 0.194)	0.431
HIV+ ART naive	-0.015 (-0.479, 0.441)		0.213 (-0.264, 0.681)	
Diabetes mellitus				
No	Ref	Ref	Ref	**Ref**
Yes	0.382 (0.251, 0.515)	< 0.0001	**0.271 (0.134, 0.405)**	**< 0.0001**
BMI	0.031 (0.023, 0.035)	< 0.0001	**0.014 (0.010, 0.036)**	**0.044**
Hip circumference	0.015 (0.012, 0.025)	< 0.0001	**-0.015 (-0.026, -0.011)**	**0.001**
Waist Circumference	0.012 (0.010, 0.020)	< 0.0001	**0.014 (0.012, 0.021)**	**< 0.0001**

3.6.3 Factors associated with HDL-C

Factors associated with total HDL-C in both univariable and multivariable linear regression models are presented in Table 3.6 below.

Table 3.6 Factors associated with HDL-C among adults in Agincourt, 2015

Covariates	Univariable analysis		Multivariable analysis	
	β-coefficients (95%CI)	***p*-value**	**β-coefficients (95%CI)**	***p*-value**
Age	-0.014 (-0.015, 0.031)	0.171	**-0.015 (-0.012, 0.014)**	**0.055**
Sex				
Female	Ref	Ref	Ref	Ref
Male	-0.012 (-0.055, 0.040)	0.697	**-0.144 (-0.263, -0.021)**	**0.018**
Exposure				
HIV negative	Ref	Ref	**Ref**	**Ref**
HIV+ ART+	0.170 (0.101, 0.240)	<0.0001	**0.172 (0.043, 0.315)**	**0.038**
HIV+ ART naive	-0.152 (-0.391, 0.099)		**-0.131 (-0.544, 0.281)**	
Smoking habits				
Never	Ref	Ref	Ref	Ref
Ever or Current	0.025 (-0.033, 0.085)	0.132	**-0.140 (-0.281, 0.015)**	**0.058**
Alcohol				
Never	Ref	Ref	**Ref**	**Ref**
Ever or Current	0.089 (0.055, 0.145)	< 0.0001	**0.194 (0.075, 0.314)**	**0.002**
Fruits	-0.019 (-0.045, 0.009)	0.159	-0.031 (-0.109, 0.011)	0.183
Waist circumference	-0.008 (-0.020, -0.001)	< 0.0001	**-0.014 (-0.020, -0.001)**	**0.001**
Visceral fat	-0.044 (-0.051,-0.025)	< 0.0001	**-0.031 (-0.044, -0.010)**	**0.002**

At the univariable level, age, sex, HIV exposure status, marital status, alcohol consumption, smoking, waist circumference, higher number of fruits servings per day and visceral fat were associated with HDL-C levels at $p < 0.2$ (Table 3.6). Apart from HIV+ participants on ART, and alcohol consumption, all the above factors were negatively correlated with serum HDL-C levels.

However at the multivariable level, sex ($p = 0.018$), HIV exposure status ($p = 0.038$), waist circumference ($p = 0.001$), alcohol consumption ($p = 0.002$) and visceral fat ($p = 0.002$) correlated with serum HDL-C levels (see Table 3.6). Smoking and age were marginally associated with HDL-C levels ($p = 0.055$ and 0.058, respectively).

3.6.4 Factors associated with LDL-C

Factors associated with LDL-C as ascertained from linear univariable and multivariable regression analyses are summarized in Table 3.7.

Table 3.7 Factors associated with LDL-C on a linear multivariable regression analysis

Covariates	Univariable analysis		Multivariable analysis	
	β-coefficients (95%CI)	***p*-value**	**β-coefficients (95%CI)**	***p*-value**
Age	0.013 (0.005, 0.013)	< 0.0001	**0.024 (0.019,0.035)**	**0.005**
Sex				
Female	Ref	Ref	Ref	Ref
Male	-0.330 (-0.419,-0.229)	< 0.0001	**-0.221 (-0.431, -0.013)**	**0.044**
Exposure				
HIV-	Ref	Ref	Ref	Ref
HIV+ART+	-0.151 (-0.294,-0.015)	0.057	-0.079 (-0.312,0.151)	0.486
HIV+ART naive	-0.290 (-0.794,0.216)		0.353 (-0.365,1.071)	
Education				
No formal education	Ref	Ref	Ref	Ref
Some primary (1-7 years)	-0.085 (-0.180,0.033)	0.021	0.034 (-0.155,0.224)	0.670
Some secondary (8-11 years)	-0.245 (-0.391,-0.090)		-0.131 (-0.411,0.140)	
Secondary or more (12+ years)	-0.032 (-0.181, 0.125)		-0.021 (-0.305, 0.264)	
Marital Status				
Currently married	Ref	Ref	Ref	Ref
Never married	-0.121 (-0.334,0.091)	0.003	0.244 (-0.111,0.590)	0.372
Separated / divorced	0.034 (-0.108,0.169)		0.163 (-0.085,0.393)	
Widowed	0.182 (0.071,0.290)		0.034 (-0.180,0.245)	
Alcohol				
Never	Ref	Ref	Ref	Ref
Ever/Current	-0.190 (-0.294,-0.109)	< 0.0001	0.121 (-0.080, 0.339)	0.235
Smoking				
Never	Ref	Ref	Ref	Ref
Ever/Current	-0.304 (-0.410, -0.199)	< 0.0001	-0.140 (-0.384, 0.112)	0.272
Waist circumference	0.009 (0.006, 0.013)	< 0.0001	**0.014 (0.010, 0.029)**	**< 0.0001**
VAT:SCAT ratio	-0.033 (-0.051, -0.014)	0.013	-0.024 (-0.051,0.015)	0.143

A large number of variables were associated with serum LDL levels at $p < 0.2$ in univariable analysis (see Table 3.7). However, after adjusting for potential confounders only age ($p = 0.005$), sex ($p = 0.044$) and waist circumference ($p < 0.0001$) were significantly associated with serum LDL-C levels. Age and waist circumference were both positively associated with LDL, whilst

males had lower LDL levels than females (see Table 3.7). VAT and SCAT dropped from the model because they interacted significantly with VAT/SCAT ratio.

3.7 Factors associated with dyslipidemia

We focused initially on the independent effect of HIV and ART status on dyslipidemia, increased TC, TGs and LDL as well as decreased HDL (see Table 3.8).

The prevalence of dyslipidemia differed significantly between exposure groups. The HIV+ ART naïve had a greater than two fold higher odds of dyslipidemia compared to HIV negative participants (OR 2.30 [0.84, 6.21]; $p = 0.008$). The HIV+ART+ participants had a reduced risk of dyslipidemia compared to HIV negative participants (OR 0.69 [0.52, 0.92]; $p = 0.009$)

HIV status was not associated with the risk of increased TC or LDL-C ($p = 0.269$ and $p = 0.660$, respectively) among study participants who were receiving ART.

The odds of increased TGs was 27% lower among HIV positive participants on ART than HIV negative participants, but just missed statistical significance (0.73 [0.51-1.04]; $p = 0.086$). The risk for high TG was higher in those HIV positive and not receiving ART when compared to HIV negative subjects but this risk was not statistically significant (1.60 [0.55-4.64]; $p = 0.148$).

HIV+ ART naïve participants had a nearly 2.5 times higher risk of low HDL than HIV negative participants (2.46, [0.94-6.43]; $p = 0.015$). The HIV+ART+ participants had a reduced risk of low HDL-C compared to HIV negative participants (OR 0.69 [0.49, 0.97]; $p = 0.031$).

In terms of gender effects, men had a significantly lower risk for both high TC ($p < 0.0001$) and high LDL (p=0.005) but had a higher risk of decreased HDL-C ($p < 0.0001$) compared to women (see Table 3.8). There was no difference in risk of dyslipidemia ($p = 0.627$) and increased triglycerides ($p = 0.109$) between men and women (see Table 3.8).

Table 3.8 Independent influence of HIV/ART status and sex on serum lipid levels among adults in Agincourt, 2015

	Odds ratios, OR (95%CI)				
	Dyslipidemia[o]	**High TC[p]**	**High TGs[q]**	**Low HDL-C[r]**	**High LDL-C[s]**
Exposure					
HIV negative	Ref	Ref	Ref	Ref	Ref
HIV+ on ART	**0.689 (0.521,0.920)****	0.691 (0.354,1.339)	0.732 (0.511,1.044)	**0.694 (0.490,0.973)***	0.764 (0.325,1.810)
HIV+ ART naïve	**2.301 (0.842,6.215)****	- -	1.602 (0.553,4.644)	**2.460 (0.940,6.431)***	1.960 (0.251,15.271)
Sex					
Women	Ref	Ref	Ref	Ref	Ref
Men	0.965 (0.790,1.155)	**0.288 (0.170,0.484)*****	0.834 (0.661,1.045)	**1.450 (1.191,1.791)*****	**0.404 (0.210,0.760)****

[o]Dyslipidemia definition: Elevated TC (≥6.21 mmol/L), AND/OR Elevated triglycerides (>2.25 mmol/L), AND/OR Low HDL (<1.19 mmol/L), AND/OR Elevated LDL (>4.1 mmol/L), OR reports of ever diagnosed with high cholesterol; [p]Elevated total cholesterol (≥6.21 mmol/L); [q]Elevated triglycerides (>2.25 mmol/L); [r]Low HDL (<1.19 mmol/L); [s]Elevated LDL (>4.1 mmol/L) (46). Level of significance denoted as ***p<0.05, **p<0.01, ***p<0.0001** vs reference group

Serum lipids levels were combined to generate a dichotomous outcome, dyslipidemia that was then used as the main outcome variable in univariable (i.e. unadjusted) and multivariate (i.e. adjusted) logistic regression analyses. The results of these analyses are presented in Table 3.9 below.

Table 3.9 Factors associated with dyslipidemia among adults in Agincourt sub-district, 2015

Factors	Unadjusted OR (95%CI)	*p*-value	Adjusted OR (95%CI)	*p*-value
Age in years	0.990 (0.978, 1.009)	0.318	0.988 (0.965, 1.004)	0.089
Sex				
Female	Ref	Ref	Ref	Ref
Male	0.960 (0.795, 1.152)	0.627	1.299 (0.981, 1.715)	0.063
Exposure status				
HIV-negative	Ref	Ref	**Ref**	**Ref**
HIV+ ART use	0.689 (0.524, 0.921)	0.009	**0.861 (0.646, 1.172)**	**0.032**
HIV+ ART naïve	2.279 (0.844, 6.210)		**3.789 (1.275, 11.302)**	
Alcohol consumption				
Never	Ref	Ref	Ref	Ref
Ever/Current	0.761 (0.630, 0.910)	0.003	0.844 (0.652, 1.081)	0.174
Smoking				
Never	Ref	Ref	Ref	Ref
Ever/Current	0.801 (0.644, 1.001)	0.055	1.054 (0.754, 1.461)	0.784
Diabetes mellitus				
No	Ref	Ref	Ref	Ref
Yes	1.914 (1.434, 2.545)	< 0.0001	**1.665 (1.202, 2.301)**	**0.002**
Waist circumference	1.034 (1.020, 1.045)	< 0.0001	**1.021 (1.010, 1.034)**	**< 0.0001**
BMI categories in kg/m^2	1.009 (1.003, 1.071)	< 0.0001	0.901 (0.952, 1.055)	0.945
Underweight	Ref	Ref	**Ref**	**Ref**
Normal weight	1.098 (0.681, 1.760)	< 0.0001	**1.006 (0.573, 1.765)**	**0.0004**
Overweight	2.174 (1.351, 3.490)		**1.851 (1.024, 3.355)**	
Obese	2.656 (1.660, 4.255)		**1.514 (0.771, 2.940)**	

Factors independently associated with a higher or lower risk of dyslipidemia included male gender, exposure status, alcohol use, smoking, diabetes, waist and BMI (Table 3.9).

After adjusting for the factors in Table 3.9 above, the following were associated with dyslipidemia in the multivariable model: exposure status, diabetes, waist circumference, BMI and age (marginally significant).

Age as a continuous variable was associated with a 2% decreased odds of dyslipidemia, however this effect missed statistical significance ($p = 0.089$). The odds of dyslipidemia among men was 29% higher than among women and this observation was marginally significant ($p = 0.063$).

The subjects who were HIV positive and ART naïve had almost a 4-fold higher risk of dyslipidemia than those who were HIV negative.

The odds of dyslipidemia increased by 2% for every 1 cm increase in waist circumference ($p<0.0001$). Increasing BMI was also associated with an increased risk of dyslipidemia.

Participants with diabetes as a co-morbidity had a 66% higher risk of dyslipidemia compared to participants without diabetes mellitus (OR 1.66 [1.34, 1.71]; $p = 0.002$).

4.0 CHAPTER 4: DISCUSSION AND CONCLUSION

In this chapter, we present an overview of significant findings from our study with possible explanations for these findings. We also compared our findings to similar studies conducted globally and in South Africa.

Due to the relatively small numbers of person with HIV+ but ART naive, a sensitivity analysis was done using the adjusted regression models with this group and with this group combined with the larger HIV+ART+ group. The results were similar hence in both analyses and therefore we present the model with the HIV+ART naive group. We however note that the results associated with this group must be interpreted with caution due to the low sample number (n).

4.1 Overview of study findings

In this study, we described the socio-demographic characteristics, behaviour, clinical history and anthropometry of adults residing in the Agincourt sub-district, rural South Africa between 2013 and 2015. We also determined differences in mean levels of serum lipids and the prevalence of dyslipidemia stratified by sex and HIV/ART status.

We looked at the individual influence of HIV/ART status on each of the serum lipids, first as continuous variables and secondly as binary outcomes. We then examined these associations adjusting for potential confounding variables measured in the study.

The major findings from this large population-based cross-sectional study include a relatively high proportion of participants over 65 years old signifying an ageing population; a high prevalence of HIV infection, being slightly higher in females; and a high prevalence of dyslipidemia across all age-sex groups.

We also observed various complex associations between persons' HIV/ART status and the four lipid fractions when applying multivariable regression models. Our findings show that HIV+ participants on ART had a lower risk of dyslipidaemia and HIV+ ART naïve were at a higher risk of dyslipidemia compared with HIV sero-negative participants.

4.2 Basic demographic characteristics of study participants.

The study population consisted of 2110 adults and was largely an ageing population with a mean age of 58.54 ± 10.91. The study participants were predominantly South African with a near 30% proportion of immigrants from Mozambique, which shares a border with the study area (66). The majority of women in the study area had never attended school while a majority of men had attained some form of primary education. This corresponds to previous authors' findings who described quality of education in the study area as poor, with limited progression from primary to secondary level (66). Nearly half of the women were married while more than half of men were married. There proportion of widowed women was higher than in men. This reflects the lower life expectancy of men compared to women – that may partly be because of poorer such due the HIV pandemic which prior to the HAART era caused high mortality especially among the male population.

While more men had full time employment, most women were likely to be homemakers. In addition to the unavailability of adequate jobs, the level of education is generally low in this age group. Wealth was distributed equally across the five key household wealth asset indices and there was no difference between men and women.

4.3 Behavioural risk factors and dietary history of study participants

Alcohol and smoking are established risk factors for cardiometabolic diseases (7). Higher proportions of men either consumed alcohol or were currently drinking alcohol compared to women. More than half of study participants had never smoked however, more men were likely to have a previous history of smoking or are currently smoking compared to females.

Previous authors had observed the study area was undergoing a transition to high rates of cardiometabolic disease with an increase in the number of cardiovascular risk factors occurring over the life course (66). Our findings confirm this increasing burden of risk factors resulting from lifestyle changes and the effects of changing economic status and urbanization (4, 51).

The median (IQR) sleeping hours per night was 9 hours (5-14). This correlated with estimates reported by previous studies (16, 79). Some meta-analysis reported sleeping ≤5–6 hours/night is associated with higher risk of cardiometabolic diseases (80). Furthermore, a study in Soweto

reported similar sleep duration among older participants and found a protective effect on increasing BMI (81).

However, in the multivariable regression analyses we saw no relationship between sleep duration and lipid levels.

Even though our study population was an ageing one, the median (IQR) MVPA exceeded the WHO recommended 300 minutes per week required to achieve additional health benefits and to reduce risk of cardiometabolic diseases (82). These finding are similar to findings from a study in Soweto, South Africa (81, 83). A recent study in the Agincourt area found a slightly higher level of MVPA among study participants than was found in the current study. This finding suggests a protective effect against cardiometabolic diseases (51).

Dietary history was measured by servings of fruits and vegetables per day as well as the consumption of sugar-sweetened beverages (SSBs) per week. On average, adults in Agincourt have one and two servings of fruits and vegetables per day, respectively. An adult in Agincourt consumes at least one can of SSB per day.

There was no difference in dietary patterns between women and men. Several reviews have reported that high intake of dietary fibre (fruits, vegetables and whole grain) lowers total cholesterol and LDL-C (84). A study in a South African population made a similar observation of a negative correlation between eating fruits and vegetable and serum lipid levels (85).

The Nutrition Society of South Africa (NSSA) recommends consumption of vegetable and fruits daily but the guidelines do not give a recommended number per day (86). However, the Agincourt population is eating too little fruit and vegetables per day as international guidelines recommend at least 5 portions of fruit and vegetables per day (87).

4.4 Clinical history and anthropometric characteristics of study participants

The prevalence of diabetes among study participants was 11.5%, which is a significant increase from the 3.0% reported by a previous study conducted in 2011 (51). However, this study included subjects less than 40 years-of-age and this might account for the lower prevalence. This co-morbidity – diabetes – is one that could contribute to dyslipidemia in the study population.

Studies have reported increased dyslipidemia among diabetics. This is attributed to defective removal of lipids from the bloodstream and changes in plasma lipoproteins resulting in increased triglycerides and decreased HDL-C (88, 89).

There was a higher prevalence of obesity in women than men and this correlated with previous findings in the study area and other South African populations (66, 68). The mean visceral adipose tissue was higher in men whilst the mean subcutaneous tissue was higher among women. This represents the expected sex distribution of central obesity as documented in the literature (90).

The prevalence of HIV in the study population was slightly higher than the national estimates in 2012 (9, 10) and a previous study conducted within the same study setting in 2013 (6). Sex distribution of HIV prevalence in our study reflects the HIV epidemiology across South Africa and sub-Saharan Africa where females than males are affected (9).

This was similar to the sex distribution observed in a recent study in the Agincourt area (51). This distribution is a sharp contrast from high-income countries where the prevalence is high among males driven mainly by special population groups such as men having sex with men (91, 92).

4.5 Lipid levels and prevalence of dyslipidemia according to gender and HIV and ART status

Our findings showed that women had a higher mean LDL-C and TC compared to males, and this correlated with findings from a study in Saudi Arabia (60) that sought to examine gender differences in prevalence of dyslipidemia.

However, we observed no difference in mean triglycerides and HDL-C levels between men and women in our study and this differed from who reported higher levels of HDL-C among men compared to women (60).

A study in urban Cape Town observed that males were more likely to have high TGs and low HDL-C while women rather had higher LDL-C and TC (32).

We observed no difference in the mean levels of TC, LDL-C and HDL-C across the three HIV/ART exposure groups. However, subjects who were HIV positive and receiving ART reported the lowest mean levels of triglycerides.

The prevalence of low HDL-C, high triglycerides and high LDL-C was highest between the HIV positive and ART naïve group. These findings correlate with many studies conducted across Africa (28, 48, 58, 59, and 93) but differ from one previous study that reported low HDL-C (62). The use of ART has previously been reported to be associated with high TC, HDL-C, LDL-C and triglycerides.

A longitudinal study of a cohort of HIV+ patients on first line ART in a South African population also demonstrated significant increases in levels of all types of lipids after 2 years of ART use (38).

We observed the overall prevalence of dyslipidemia among study participants was 43.7% with men having a higher prevalence compared to women. We further observed a higher prevalence of dyslipidemia among HIV+ but ART naive participants (64.7 [40.4, 83.2] %) compared to HIV+ on ART (35.8 [30.0, 42.0] %) and HIV negative subjects (44.5 [42.0, 47.1] %).

These findings were similar to those reported in South Africa (32, 51, and 59), East Africa (31, 41, and 93) and West African (28, 48, and 58).

4.6 Factors associated with serum lipids: data from multivariable regression models

Age and sex had varied associations with each of the four serum lipid fractions. Age was associated with increased levels of TC, triglycerides, LDL-C and a decrease in HDL-C, whilst male gender, independent of anthropometric measures, was observed to be negatively correlated with all four species of lipids. This is consistent with other studies that reported a higher risk of increased TC and higher HDL-C among females (94)

Age has been reported as one of the non-modifiable risk factors for atherogenic lipid levels (1). Though the mechanism is unclear, it has been reported that increasing age is associated with

increased production of LDL-C, which occurs in parallel with a decreased clearance from the body (94).

Although age is associated with increasing cholesterol levels this can be countered by behavioural or drug therapy, with intervention studies demonstrating that lipid levels can be modified with dietary and lifestyle interventions (94).

Factors associated with TC included level of education, alcohol consumption, diabetes and BMI. Our results showed that there was a tendency (p = 0.093) for higher educational level to be associated with decreased levels of TC. Studies conducted in America have reported a similar effect (95).

A possible explanation for this could be that educated participants are likely to have a better perception of risk, better health seeking behaviour, better compliance with medication and better access to information on interventions on modifiable risk factors.

In our study, we observed that alcohol consumption was associated with increased TC levels and that nearly two-thirds of the population consumed alcohol, however we were unable to quantify and characterise the drinking patterns.

Alcohol consumption was also associated with raised HDL levels. Alcohol consumption has been reported to cause increases in TC, LDL-C, triglyceride and HDL-C (2). A study among alcoholics in America reported elevated levels of all subclasses of HDL-C (96). This is likely because alcohol in moderate and high quantities causes an increase in the transport rate of apolipoproteins A-1 and A-2 (97).

Increasing BMI was associated with increases in TC and triglycerides. These associations have been reported by previous epidemiological studies (94, 98). The major drawback to these studies has been under representation of obese subjects. Our study had a high proportion of obese participants and our findings correlate with several case-control and cross-sectional studies (59, 85, 94, 98). Waist circumference was positively correlated with serum lipids. After adjusting for BMI in the models, waist circumference was strongly correlated with increased triglycerides and LDL-C and decreased HDL-C. Such associations have previously been reported in a number of other studies (81, 94, 99, 100).

We also observed that in adjusting for waist and BMI, increased hip circumference was associated with decreased serum triglyceride. This is contrary to a study in an Asian population (101) but correlates with studies from Australia (102) and Canada (103).

Hip adipose tissue is reported have a better buffering effect on lipid-excess than abdominal fat. This buffering effect is attributed to the number and activity of mitochondria in these adipocytes (104, 105). A study corroborated these findings when they observed that women with high hip fat were more efficient at storing fat derived from a meal than women with high visceral fat (106).

Precise measures of central obesity such as ultrasound measurements of visceral and subcutaneous adipose tissue were novel to our study since previous studies in Africa have not made use of these measures. Our findings show that visceral adipose tissue was associated with decreased serum HDL-C levels. This is consistent with studies in Caucasian populations, which report an association between visceral adipose tissue and dyslipidemia among HIV+ men and women (44, 54).

Tobacco (smoke phase) and its products such as tar has been reported to cause increased oxidative stress resulting in lipid peroxidation (resulting in increased LDL-C and decreased HDL-C) (107). Consistent with this observation was our finding that smoking tended ($p=0.058$) to be associated with decreased levels of HDL-C.

We observed that being HIV+ and on ART was independently associated with an increase in HDL-C levels. This was largely consistent with findings from studies across sub-Saharan Africa that report increased levels of TC, triglycerides, LDL-C and HDL-C in subjects receiving ART (4, 38).

However, our results differ from these studies in that we did not observe higher TC, LDL-C or triglyceride levels in subjects receiving ART. The reason for this is not clear. However, we can hypothesize that subjects receiving ART are accessing health care facilities more frequently than those who are HIV negative or ART naïve.

They may therefore be receiving better clinical care, especially in the sub-district of Agincourt, which is part of a large health and demographic surveillance system.

A study in a rural HIV clinic in Limpopo reported better health outcomes with improved access to healthcare. Even though this study did not assess dyslipidemia as one of the outcomes, high patient retention due to access to healthcare was associated with good treatment outcomes (108).

In addition, this is an older population and it is feasible that ART therapy may not be as atherogenic as in younger subjects. These hypotheses obviously require further investigation.

Diabetes was associated with increased total cholesterol and triglyceride levels. These findings are similar to a study conducted in Cape Town (109) and a recent study in the Agincourt area (51). Furthermore, diabetes is a well-known cause of dyslipidemia (88, 110).

In our study we have therefore demonstrated the influence of age, sex, waist, hip, BMI, alcohol consumption, diabetes and education on the various species of serum lipids.

We have in this study observed that ART use was associated with increased HDL-C levels whilst there was no association with TC, LDL-C and triglyceride levels in the multivariable regression models.

4.7 Factors associated with dyslipidemia

We initially dichotomised each of the serum lipid species and examined the independent risk of dyslipidemia and high levels of TC, triglycerides, LDL-C and low levels of HDL-C in each of the three HIV/ART groups.

Similar to previous studies (31, 32, 51), we observed that HIV positive patients who were ART naïve had a greater odds of dyslipidemia, high TGs and LDL-C and low HDL-C when compared to HIV negative patients. However, only the risk of low HDL levels was statistically significant.

We further noticed that HIV+ART+ participants had a reduced risk of dyslipidemia and of low HDL.

After adjusting for several confounders, we observed that dyslipidemia was associated with age, HIV/ART status, diabetes, waist circumference and BMI. Sex was marginally associated with an increased risk of dyslipidemia with males having a higher risk than females as observed in other studies (5).

With or without ART, HIV infection is generally associated with dyslipidemia (18). In our study, HIV+ART naïve participants had a higher risk of dyslipidemia compared to HIV negative participants. This has been reported in studies conducted in West Africa (111) South Africa (32) and non-African countries (27). This can be explained by the fact that as a population based study, most of the HIV+ART naïve may be in their initial phase of high viremia, which is associated with abnormal lipid metabolism (112).

Consistent with other studies, increased BMI and waist circumference, and diabetes were all associated with dyslipidemia (111, 112) It is interesting that BMI and waist independently affected lipid levels suggesting that they act via different mechanisms.

4.8 Limitations

Our study has several limitations. The cross-sectional nature of the study limited our ability to establish causal relationships between HIV/ART status and dyslipidemia. We also relied on self-reported use of ART hence; our estimated association could be biased. We did not ascertain the duration and treatment regimen of study participants. The scope of confounding variables did not include CD4+ count, viral load and inflammatory markers, which cause metabolic and body composition abnormalities (19, 29).

Furthermore, dietary intake was not studied in detail, and it is known that lipid levels are strongly influenced by diet. In addition, the sample number of subjects with HIV who were ART naïve was low, limiting our ability to identify significant effects of untreated viremia. The use of lipid lowering drugs could also affect our estimate of dyslipidemia and mask the effect of HIV and ART on lipid levels. The use of point-of-care lipids is also a limitation for the accurate estimation of lipid levels. The HIV+ ART naive group had a small number of subjects and therefore interpretation of data from this group should be made with caution.

4.9 Strengths

To our knowledge, this is the first large-scale population-based study comparing serum lipid levels among HIV negative and HIV positive participants at the population level in sub-Saharan Africa. The study, additionally, explored the influence of a more precise measure of central obesity (ultrasound measures of VAT and SCAT). This to our knowledge is the first to be used in

an Africa population of this size and with a high prevalence of HIV infection. Though not exhaustive, we had a wide range of confounding variables to enable us to examine the multiple influences on lipid levels. The sample size provided us with adequate power to answer the research question.

4.10 Conclusions

This population has a high prevalence of HIV among older adults in Agincourt. This HIV prevalence mirrors the national prevalence and presents a typical picture of HIV epidemiology in eastern and southern African regions. In this population, HIV/ART status mainly influences HDL levels with ART use associated with higher HDL and untreated HIV infection linked to lower HDL levels and a greater risk of dyslipidemia.

This cohort has a risk of dyslipidemia driven by prevailing traditional risk factors such as obesity and diabetes. With the increasing lifespan of PLWH and associated increase in co-morbidities, their long-term impact on screening, prevention, and treatment of dyslipidemia needs to be better understood. Health systems must strengthen to embrace these evolving health challenges.

4.11 Recommendations

With the advent of ART, more patients are likely to go through the normal ageing process and experience more metabolic disorders like dyslipidemia. We therefore recommend a longitudinal cohort to evaluate the effects of HIV and ART use on serum lipid levels and associated cardiometabolic diseases. The HAALSI/AWI-Gen collaborative studies is timely offers a unique platform to study the evolution of long-term cardiometabolic risk in an ageing population with a high burden of HIV.

We also recommend routine screening and treatment of disorders of serum lipids among the HIV patients to help decrease the risk of cardiovascular diseases and to increase ART coverage. Further research questions should focus on the effects of ART drug regimens, especially newer versus older drugs, on lipid levels.

The strong influence of obesity on lipid levels also suggests that lifestyle interventions should be used to reduce the risk of dyslipidemia and the associated cardiometabolic diseases.

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APPENDIX 1: Plagiarism Declaration Form

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I **Engelbert Adamwaba Nonterah** (Student number: **1404786**) am a student registered for the degree of **MSc Epidemiology** in the academic year **2017**.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

Signature:  Date: **19th June, 2017**

APPENDIX 2: Ethics Clearance Certificate

R14/48 Dr Engelbert Adamwaba Nonterah et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M161197

NAME: (Principal Investigator)	Dr Engelbert Adamwaba Nonterah et al
DEPARTMENT:	School of Public Health, Epidemiology and Biostatistics MRC/Wits Rural Public Health and Health Transitions Research Unit (Agincourt) Sydney Brenner Institute of Molecular Bioscience
PROJECT TITLE:	The Effects of HIV and ART on Serum Lipids among Adults in Agincourt in 2015
DATE CONSIDERED:	25/11/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Nigel Crowther
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	30/11/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Philip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in November and will therefore be due in the month of November each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 01/12/2016

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 3: Sensitivity analyses with the HIV+ ART- group and with the group combined with the HIV+ART+ group.

Table 4: Comparison of adjusted linear regression models for TC in which the HIV+ART- is present as an individual group or is combined with the HIV+ART+ group

Covariates	ANALYSIS WITH THE HIV+ ART- GROUP (Adjusted analysis)		ANALYSIS WITHOUT THE HIV+ ART- GROUP (Adjusted analysis)	
	β-coefficients (95%CI)	**P-value**	**β-coefficients (95%CI)**	**P-value**
Age	**0.024 (0.010, 0.031)**	**0.014**	**0.018 (0.010, 0.025)**	**0.016**
Sex				
Female	Ref	Ref	Ref	Ref
Male	**-0.315 (-0.572, -0.051)**	**0.019**	**-0.291 (-0.553, -0.034)**	**0.030**
Exposure status				
HIV negative	Ref	Ref	Ref	Ref
HIV+ ART use	0.014 (-0.264, 0.279)	0.684	0.021 (-0.253, 0.291)	0.897
HIV+ ART naive	0.412 (-0.510, 1.329)		-	-
Education Status				
No formal education	Ref	Ref	Ref	Ref
Some primary (1-7 years)	-0.081 (-0.291, 0.135)	0.093	-0.090 (-0.310, 0.122)	0.074
Some secondary (8-11 years)	-0.230 (-0.541, 0.074)		-0.241 (-0.552, 0.060)	
Secondary or more (12+ years)	-0.394 (-0.720, 0.075)		-0.412 (-0.742, -0.091)	
Marital Status				
Currently married	Ref	Ref	Ref	Ref
Never married	0.304 (-0.092, 0.691)	0.289	0.291 (-0.091, 0.691)	0.330
Separated/Divorced	0.195 (-0.080, 0.461)		0.189 (-0.090, 0.455)	
Widowed	0.016 (-0.235, 0.255)		0.015 (-0.243, 0.254)	
Diabetes mellitus				
No	Ref	Ref	Ref	Ref
Yes	**0.310 (0.019, 0.614)**	**0.039**	**0.310 (0.015, 0.612)**	**0.040**
Alcohol consumption				
Never	Ref	Ref	Ref	Ref
Ever or current	**0.250 (0.021,** 0.480**)**	**0.038**	**0.225 (-0.014, 0.463)**	**0.063**
Smoking History				
Never	Ref	Ref	Ref	Ref
Ever or current	-0.235 (0.524, 0.055)	0.102	-0.234 (-0.513, 0.055)	0.106
Body mass index	**0.024 (0.018, 0.041)**	**0.030**	**0.024 (0.015, 0.043)**	**0.035**
Hip circumference	-0.013 (-0.021, 0.015)	0.160	-0.013 (-0.024, 0.027)	0.167
VAT:SCAT ratio	-0.013 (-0.041, 0.022)	0.649	-0.015 (-0.041, 0.025)	0.636

Table 5: Comparison of adjusted linear regression models for TGs in which the HIV+ART- is present as an individual group or is combined with the HIV+ART+ group

Covariates	ANALYSIS WITH THE HIV+ ART- GROUP (Adjusted analysis)		ANALYSIS WITHOUT THE HIV+ ART- GROUP (Adjusted analysis)	
	β-coefficients (95%CI)	**P-value**	**β-coefficients (95%CI)**	**P-value**
Age	**0.013 (0.011, 0.035)**	**0.003**	**0.013 (0.010, 0.026)**	**0.003**
Sex				
Female	Ref	Ref	Ref	Ref
Male	**-0.099 (-0.198, 0.010)**	**0.053**	-0.103 (-0.190, 0.016)	0.061
Exposure status				
HIV-	Ref	Ref	Ref	Ref
HIV+ ART use	0.065 (-0.060, 0.194)	0.431	0.066 (-0.060, 0.195)	0.306
HIV+ ART naive	0.213 (-0.264, 0.681)		-	-
Diabetes mellitus				
No	Ref	**Ref**	Ref	Ref
Yes	**0.271 (0.134, 0.405)**	**< 0.0001**	**0.273 (0.130, 0.415)**	**< 0.0001**
BMI	**0.014 (0.010, 0.036)**	**0.044**	0.012 (-0.019, 0.025)	0.067
Hip circumference	**-0.015 (-0.026, -0.011)**	**0.001**	**-0.009 (-0.080, -0.010)**	**0.002**
Waist Circumference	**0.014 (0.012, 0.021)**	**< 0.0001**	**0.021 (0.010, 0.026)**	**< 0.0001**

Table 6: Comparison of adjusted linear regression models for HDL in which the HIV+ART- is present as an individual group or is combined with the HIV+ART+ group

Covariates	ANALYSIS WITH THE HIV+ ART- GROUP (Adjusted analysis)		ANALYSIS WITHOUT THE HIV+ ART- GROUP (Adjusted analysis)	
	β-coefficients (95%CI)	**P-value**	**β-coefficients (95%CI)**	**P-value**
Age	-0.015 (-0.012, 0.014)	0.055	0.012 (-0.021, 0.018)	0.059
Sex				
Female	Ref	Ref	Ref	Ref
Male	**-0.144 (-0.263, -0.021)**	**0.018**	**-0.152 (-0.271, -0.036)**	**0.013**
Exposure				
HIV negative	**Ref**	**Ref**	Ref	Ref
HIV+ ART+	**0.172 (0.043, 0.315)**	**0.038**	**0.171 (0.035, 0.302)**	**0.016**
HIV+ ART naive	**-0.131 (-0.544, 0.281)**		-	-
Smoking habits				
Never	Ref	Ref	Ref	Ref
Ever or Current	**-0.140 (-0.281, 0.015)**	**0.058**	**-0.144 (-0.291, -0.015)**	**0.050**
Alcohol				
Never	**Ref**	**Ref**	Ref	Ref
Ever or Current	**0.194 (0.075, 0.314)**	**0.002**	**0.191 (0.074, 0.325)**	**0.002**
Fruits	-0.031 (-0.109, 0.011)	0.183	-0.031 (-0.080, 0.024)	0.248
Waist circumference	**-0.014 (-0.020, -0.001)**	**0.001**	**-0.040 (-0.107, -0.010)**	**0.008**
Visceral fat	**-0.031 (-0.044, -0.010)**	**0.002**	**-0.032 (-0.056, -0.010)**	**0.002**

Table 7: Comparison of adjusted linear regression models for LDL in which the HIV+ART- is present as an individual group or is combined with the HIV+ART+ group

Covariates	ANALYSIS WITH THE HIV+ ART- GROUP (Adjusted analysis)		ANALYSIS WITHOUT THE HIV+ ART- GROUP (Adjusted analysis)	
	β-coefficients (95%CI)	**P-value**	**β-coefficients (95%CI)**	**P-value**
Age	**0.024 (0.019,0.035)**	**0.005**	**0.023 (0.010,0.034)**	**< 0.0001**
Sex				
Female	Ref	Ref	Ref	Ref
Male	**-0.221 (-0.431, -0.013)**	**0.044**	-0.240 (-0.475, 0.015)	0.051
Exposure				
HIV-	Ref	Ref	Ref	Ref
HIV+ART+	-0.079 (-0.312,0.151)	0.486	-0.020 (-0.261, 0.263)	0.576
HIV+ART naive	0.353 (-0.365,1.071)		-	-
Education				
No formal education	Ref	Ref	Ref	Ref
Some primary (1-7 years)	0.034 (-0.155,0.224)	0.670	0.024 (-0.171, 0.206)	0.688
Some secondary (8-11 years)	-0.131 (-0.411,0.140)		-0.140 (-0.420, 0.135)	
Secondary or more (12+ years)	-0.021 (-0.305, 0.264)		-0.061 (-0.357, 0.220)	
Marital Status				
Currently married	Ref	Ref	Ref	Ref
Never married	0.244 (-0.111,0.590)	0.372	0.230 (-0.134, 0.580)	0.416
Separated / divorced	0.163 (-0.085,0.393)		0.161 (-0.080, 0.394)	
Widowed	0.034 (-0.180,0.245)		0.032 (-0.180, 0.244)	
Alcohol				
Never	Ref	Ref	Ref	Ref
Ever/Current	0.121 (-0.080, 0.339)	0.235	0.055 (-0.190, 0.295)	0.678
Smoking				
Never	Ref	Ref	Ref	Ref
Ever/Current	-0.140 (-0.384, 0.112)	0.272	-0.070 (-0.371, 0.205)	0.591
Waist circumference	**0.014 (0.010, 0.029)**	**< 0.0001**	**0.010 (0.009, 0.075)**	**0.003**
VAT:SCAT ratio	-0.024 (-0.051,0.015)	0.143	-0.010 (-0.040, 0.025)	0.684

Table 8: Independent influence of HIV/ART status and sex on serum lipid levels among adults in Agincourt, 2015

Exposure	**Odds ratios, OR [95%CI]** ***p*** **- value**				
	Dyslipidemia	**High TC**	**High TGs**	**Low HDL-C**	**High LDL-C**
With HIV+ART-					
HIV negative	Ref	Ref	Ref	Ref	Ref
HIV+ on ART	**0.689 [0.521,0.920] *p* = 0.011**	0.691 [0.354,1.339] *p* = 0.269	0.732 [0.511,1.044] *p* = 0.086	**0.694 [0.490,0.973] *p* = 0.031**	0.764 [0.325,1.810] *p* = 0.541
HIV+ ART naïve	2.301 [0.842,6.215] *p* = 0.105	-	1.602 [0.553,4.644] *p* = 0.385	**2.460 [0.940,6.431] *p* = 0.056**	1.960 [0.251,15.271] *p* = 0.519
Without HIV+ART-					
HIV negative	Ref	Ref	Ref	Ref	Ref
HIV+ on ART	**0.694 [0.523, 0.925] *p* = 0.011**	0.689 [0.354, 1.345] *p* = 0.246	0.733 [0.511, 1.047] *p* = 0.086	**0.694 [0.491, 0.976] *p* = 0.031**	0.765 [0.324, 1.812] *p* = 0.541
HIV+ ART naïve	-	-	-	-	-

Table 9: Comparison of adjusted logistic regression models for dyslipidemia in which the HIV+ART- is present as an individual group or is combined with the HIV+ART+ group

Factors	ANALYSIS WITH THE HIV+ ART- GROUP		ANALYSIS WITHOUT THE HIV+ ART- GROUP	
	Adjusted OR (95%CI)	**P-value**	**Adjusted OR (95%CI)**	**P-value**
Age in years	0.988 (0.965, 1.004)	0.089	0.990 (0.980, 1.005)	0.156
Sex				
Female	Ref	Ref	Ref	Ref
Male	1.299 (0.981, 1.715)	0.063	1.291 (0.980, 1.705)	0.065
Exposure status				
HIV-negative	**Ref**	**Ref**	**Ref**	**Ref**
HIV+ ART use	**0.861 (0.646, 1.172)**	**0.032**	**0.860 (0.631, 0.970)**	**0.035**
HIV+ ART naïve	**3.789 (1.275, 11.302)**			
Alcohol consumption				
Never	Ref	Ref	Ref	Ref
Ever/Current	0.844 (0.652, 1.081)	0.174	0.832 (0.652, 1.085)	0.169
Smoking				
Never	Ref	Ref	Ref	Ref
Ever/Current	1.054 (0.754, 1.461)	0.784	1.080 (0.781, 1.505)	0.638
Diabetes mellitus				
No	Ref	Ref	**Ref**	**Ref**
Yes	**1.665 (1.202, 2.301)**	**0.002**	**1.642 (1.191, 2.290)**	**0.003**
Waist circumference	**1.021 (1.010, 1.034)**	**< 0.0001**	**1.024 (1.010, 1.045)**	**< 0.0001**
BMI categories in kg/m^2	0.901 (0.952, 1.055)	0.945		
Underweight	**Ref**	**Ref**	Ref	Ref
Normal weight	**1.006 (0.573, 1.765)**	**0.0004**	**1.020 (0.581, 1.805)**	**0.0004**
Overweight	**1.851 (1.024, 3.355)**		**1.860 (1.025, 3.390)**	
Obese	**1.514 (0.771, 2.940)**		**1.494 (0.760, 2.936)**	