SCHOOL OF POSTGRADUATE STUDIES

PLASMA FAT PREDICTORS OF IMMUNOLOGICAL, VIROLOGICAL AND CLINICAL STATUS IN ADULTS WITH HIV/AIDS IN NDOLA DISTRICT OF ZAMBIA: A CROSS SECTIONAL STUDY

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Declaration

I Christopher Nyirenda declare that this doctoral thesis represents my own work and that it has not been previously submitted in whole or in part, in any application for a degree or professional qualification at this or another University. Except where specified by reference or acknowledgement, the work presented is solely mine.

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Dedication

First and foremost I dedicate my doctoral thesis to Almighty God for his love and all sufficient grace upon my life and for enabling me to successfully complete this research project. I further dedicate this work to my loving and caring parents, late dad, Mr. Ngwire Zerah Nyirenda (MHSRIEP) and my dear mum, Mrs. Miriam Chansa Nyirenda for their various unwavering support to ensure success throughout my academic pursuits. Sincere gratitude to my wife Catherine for her spiritual, moral and material support and our children, Grace, Chileleko and Hope who though being that young have been there for me, providing moral and spiritual motivation all the way.

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Acronyms used:

AA	Arachidonic Acid
AIDS	Acquired Immunodeficiency Syndrome
ALA	Alpha Linolenic Acid
ART	Anti-retroviral Therapy
BMI	Body Mass Index
CBU	Copper belt University
CD4+	Cluster of Differentiation 4
CDC	Centre for Disease Control and Prevention
CIDRZ	Center for Infectious Disease Research in Zambia
CRP	C - Reactive Protein
DHA	Docosahexaenoic Acid
DPA	Docosapentanoic Acid
EPA	Eicosapentanoic Acid
GCP	Good Clinical Practice
HAART	Highly Active Anti-retroviral Therapy
HDL-C	High Density Lipoprotein Cholesterol
HIV	Human Immunodeficiency Syndrome
LA	Linoleic Acid
LDL-C	Low Density Lipoprotein Cholesterol
MOH	Ministry of Health
NAIDS	Nutritionally Acquired Immune Deficiency Syndrome
NTH	Ndola Teaching Hospital
OpIn	Opportunistic Infections
PEPFAR	President's Emergency Plan for AIDS Relief
PUFA	Polyunsaturated Fatty Acids
TDRC	Tropical Diseases Research Centre
UNAIDS	Joint United Nations Programme on HIV/AIDS
WHO	World Health Organization

Operational definitions of terms:

The following definitions will apply in this study:

Adults: Men and Women aged 18years and above

AIDS: Acquired Immunodeficiency Syndrome, refers to a syndrome caused by the virus called HIV

CD4+ T cells: The white blood cells that fight infection and are also the main targets for HIV

Clinical Status: Shall be the presence or absence of opportunistic infections and/or co-morbidities

FAT: A combination of total cholesterol, triglycerides and fatty acids

HDL-c: A sub-type of cholesterol also referred to as good cholesterol

HIV: Human Immunodeficiency Virus, is a virus that causes AIDS

Immunological Status: The level of immunity with regard to the CD4+ count

LDL-c: A sub-type of cholesterol also referred to as bad cholesterol

n-3 PUFA: A combination of alpha-linolenic acid, eicosapentanoic acid, decosapentanoic acid and decosahexanoic acid in the plasma

n-6 PUFA: A combination of linoleic acid and arachidonic acid in the plasma

Plasma: The liquid portion of blood that remains after red blood cells, white blood cells, platelets and other cellular elements are removed.

UFA sub-type: Referring to either n-3 PUFA or n-6 PUFA

Total PUFA: A combination of n-3 and n-6 plasma polyunsaturated fatty acids

Viral load: The amount of HIV in the blood

Abstract

Background: HIV remains a major cause of morbidity and mortality in Zambia. Nutritional imbalances in the form of micro or macro-nutrient deficiencies or excesses reported with advanced HIV disease have been associated with higher risks of HIV disease progression and mortality. This study seeks to explore the potential role of plasma fat in immune mechanisms, viral suppression and clinical status in HIV/AIDS.

Methods: We conducted a cross sectional study targeting 174 adult HIV/AIDS patients recruited and followed over a period of 12 months at a University Teaching Hospital. Participants were subjected to clinical assessments with anthropometry, CD4+ count,viral load and plasma fat measurements at baseline and repeated on a follow-up visit.

The main independent variables were total cholesterol, and its subtypes, triglyceride and BMI while the main outcome variables were CD4 count, viral load and opportunistic infection. Quantitative methods were applied to determine the relationship between the independent and outcome variables. The Wilcoxon rank sum test for continuous variables and the Chi square test for categorical variables were applied to compare the study population by gender. The multiple linear regression was applied to determine associations between the independent and dependent variables.

Results

Plasma fat types were not significantly predictive of CD4+ count, viral load or clinical status. However, we found a significant positive association between CD4+ count and the body mass index in both the unadjusted [Coef= 0.05; 95% CI (0.03, -0.08), p=0.00] and adjusted [Coef=0.04; 95% CI (0.00, 0.07), p=0.03] estimates respectively. Similarly, a significant positive association between CD4+ count and the body mass index [Coef= 0.04; 95% CI (0.00, 0.08), p=0.04] were reported for the female gender. A statistically significant inverse association was also found between absolute CD4+ count and viral load in both the unadjusted [Coef=-0.10; 95% CI (-0.14, -0.05), p=0.00] and adjusted [Coef=-0.09; 95% CI (-0.14, 0.03), p=0.01] models respectively.

Alcohol status was positively associated with viral load [Coef=1.99; 95% CI (0.39, 3.60), p=0.02], while BMI was found to be an independent predictor of opportunistic infections [Coef=-0.01; 95% CI (-0.03, 0.00), p=0.04].

Conclusion: Plasma fat was not significantly predictive of CD4+ count, viral load or clinical status. However, the consistent positive association between Plasma fat subtypes and CD4 count in the overall and the by gender models may favor a potential predictive role. There was also a by gender consistent inverse interaction between total cholesterol and opportunistic infections. Body mass index was found to be a positive predictor of CD4+ count more so by female gender and an independent predictor of prevailing opportunistic infections. Further, there was an overall and by gender consistent inverse association between BMI and viral load.

Keywords: HIV/AIDS, Plasma Fat, BMI, CD4+ count, Viral Load, Opportunistic Infections

Chapter One: Background of the Study

1.0 Introduction

In this section, the researcher will identify and describe the history and nature of the research problem. The researcher will indicate the root of the problem under investigation, appropriate context of the research problem in relation to theory, research, and/or practice, its scope, and the extent to which past studies have successfully investigated the problem, highlighting in particular, where the gaps exist which this study will attempt to narrow (Nsenduluka E, 2017).

1.1 Background to the Problem

Over the past thirty years, Zambia has made significant progress and achievements in the fight against HIV/AIDS. This has been possible as a result of the advances made in the administration of combination anti-retroviral therapy (cART), adjunct therapy and monitoring services such as CD4 count and viral load in most treatment centers (PEPFAR, 2017: 39,51).

The prevalence of HIV among adults aged 15-59 years in Zambia is 12.0% of which 14.6% among females and 9.3% among males. This corresponds to approximately 960,000 people living with HIV (PLHIV) aged 15-59 years in Zambia. Among people living with HIV aged 15-59 years who know their HIV status, 87.1% self-reported current use of ART and/or had detectable ARVs in their blood (MOH, 2019). The HIV prevalence has been declining over time falling by 19% between 2003 and 2015 (PEPFAR, 2016: 1) and a further decline suggested from 12.9% reported in 2016 (UNAIDS, 2016: 1) to the current 12%.

The Zambian government has taken further positive responses to address the HIV epidemic by implementing measures through the “prevention to care continuum” strategy in the fight against HIV (Zambia Consolidated Guidelines, 2017). The government recognizes that HIV prevention efforts must be complemented by care and support initiatives and thus has taken steps to provide antiretroviral therapy to all people living with HIV in Zambia.

This is in line with the UNAIDS targets of “90-90-90,” which are expected to bring the country closer to achieving universal access to HIV voluntary counseling, testing, treatment and care and ending AIDS as a public threat (Zambia Consolidated Guidelines, 2017). Despite the reported positive outcomes in the management of HIV/AIDS in Zambia, the HIV prevalence is still very high and HIV remains a major cause of morbidity and mortality in Zambia (PEPFAR, 2016:5).

HIV/AIDS often presents with a wide range of opportunistic infections and co-morbidities which if left unattended can result in mortality. Therefore, the role of ART alone in the management of HIV/AIDS cannot be adequate without providing appropriate adjunct therapy in the course of care.

Previous studies have revealed that low CD4 counts, advanced WHO clinical stage, anemia, immune reconstitution inflammatory syndrome and malnutrition have been associated with high early mortality in the course of treatment (Stringer J S A et al, 2006, Heimburger D C et al, 2010, Muller M et al, 2010, Ngu J N et al, 2010).

Further, a review of HIV infected adults starting ART in Lusaka Zambia found 33% of patients were undernourished by World Health Organization criteria (e.g., a body mass index (BMI)< 18.5 kg/m2), and 9% were severely malnourished (BMI <16 kg/m2) (Koethe J R, et al, 2010)**.** Studies have shown that people living with HIV/AIDS develop multiple nutrient deficiencies, both micro- and macronutrients. Micronutrient deficiencies which are commonly observed with advanced HIV disease have been associated with higher risks of HIV disease progression and mortality (Drain P K, et al, 2007). Prior research on micronutrients has quite extensively covered the role of multiple vitamins and minerals (Scrimshaw N S, et al, 1997, Cunningham R S, et al 2005, Benedich A, et al 1988, Ferencik M, et al, 2003), but the role of macronutrients such as fats in immune mechanisms involving CD4+ counts and viral load suppression in HIV/AIDS patients in resource limited settings like Zambia is not well known.

Fat is defined chemically as triglycerides; trimesters of glycerol with several fatty acids (Banik S et al., 2014, Brouwer et al., 2010).

There are abundant diverse kinds of fats, but each is a distinction on the identical chemical arrangement. More specifically, the entire fats are sources of fatty acids and glycerol (Banik S et al., 2014). Cholesterol is a waxy like substance that is found in the fat cells of our body and in the bloodstream. There are largely two types of cholesterol; one is HDL (high density lipoproteins) and another is LDL (low density lipoproteins) cholesterol (Brouwer et al., 2010). When you have your cholesterol checked, the doctor typically gives you levels of three fats found in the blood: LDL, HDL and triglycerides. In human plasma alone however, researchers have identified some 600 different types of fats or lipids relevant to our health (NIH, 2013). HDL cholesterol is also known as “good cholesterol” and LDL cholesterol is considered to be “bad cholesterol”. Higher levels of HDL protects against heart attack and stroke because it keeps LDL cholesterol from building up around the heart (Banik S and Hossain MS, 2014).

Triglycerides, cholesterol and other essential fatty acids--the scientific term for fats the body can't make on its own, store energy, insulate us and protect our vital organs. They act as messengers, helping proteins do their jobs. They also start chemical reactions involved in growth, immune function, reproduction and other aspects of basic metabolism. Fats help the body stockpile certain nutrients as well. The so-called "fat-soluble" vitamins--A, D, E and K are stored in the liver and in fatty tissues (NIH, 2013).

Fatty acids are molecules that are long chains of lipid-carboxylic acid found in fats and oils in cell membranes as a component of phospholipids and glycolipids. An essential fatty acid is a polyunsaturated fatty acid needed by the body that is synthesized by plants but not by the human body and is therefore a dietary requirement (Davis CP, 2021).

This study will focus mainly on the role of total cholesterol, LDL-c, HDL-c and triglycerides in immune mechanisms and viral load suppression in HIV while the role of fatty acids will be considered in subsequent analyses.

In this study we anticipate that early in the course of anti-retroviral therapy subjects would have enhanced appetite and corresponding food intake (Weiser SD, et al, 2010).

In this case we expect that the fat uptake from the diet would also be enhanced. Research has shown that while food supply and macronutrient intake do not necessarily change in African patients with HIV who start ART, an alternative pathophysiology is conceivable. HIV enteropathy which is common in patients with HIV disease impairs gastrointestinal function and nutrient absorption (Call SA, et al, 2000). With rapid reductions in viral load after ART is instituted, improvements in HIV enteropathy occur even in the first week of ART (Kotler DP, et al 2005). This would result in increased absorption of several nutrients including fat. It is further anticipated that with the initiation of cART, prevalence and incidence of the gastrointestinal associated opportunistic infection would reduce, thereby further minimizing potential losses of fat for example through vomiting and diarrhea.

Apart from one study that was conducted to look at polyunsaturated fatty acid (PUFA) in relation to dietary intake and cardiovascular risk factors in HIV/AIDS, (Nyirenda C K, et al, 2015), there has been no study conducted to examine the role of fat in relation to the immunological and virological status in HIV/AIDS patients in Zambia.

1.2 Statement of the Problem

Despite the progress made in the administration of combination anti-retroviral therapy (cART) and accompanying support services, the benefits in optimally improving clinical outcomes in HIV/AIDS care, have not been fully realized. Although Zambia recorded a significant decline in HIV prevalence by 19% between the years 2003 and 2015 (PEPFAR, 2016) and further decline thereafter from about 12.9% to the current 11.1% (MOH, 2019, USAID, 2022), the HIV prevalence is still very high and suggestive of more work that is needed to be done if the country has to meet the ambitious UNAIDS target of achieving the goal of universal access to ARV drugs, treating and preventing HIV, and ultimately ending the HIV epidemic by 2030.

The problem thus is that there is still evidently a high HIV prevalence coupled with a high burden of malnutrition (Koethe JR, et al, 2010) among the HIV infected adults presenting for care in Zambia. The vicious interaction between HIV/AIDS and malnutrition can have a devastating impact on clinical outcomes resulting in worsening morbidity and mortality rates. Further, most prior research work in Zambia has examined and suggested the beneficial role of micronutrients such as vitamin A, C, E, Zinc and Selenium in the management of disease in both the HIV and non-HIV setting (CIGNIS Study Team, 2010, Fiedler JL and Lividini K, 2014, Filteau S, et al, 2015, Nyati M, et al, 2016, Zyambo K, et al, 2022). The role of macro-nutrients such as fats in the management of HIV/AIDS has not been fully explored.

Notably, there has been remarkable progress toward the achievement of the UNAIDS 90-90-90 targets in adults, with 71.4% of people living with HIV aged 15-59 years diagnosed, 87.1% of those diagnosed receiving ART, and 89.2% of those on treatment with suppressed viral loads. However, it is concerning that viral load suppression is relatively lower in the younger populations living with HIV, such that only 72.6% of those aged 15-24 years who were on treatment had a suppressed viral load (MOH, 2019).

The suggested sub-optimal trend in immunological response among some patients despite receiving treatment further signals the need for additional strategies to improve clinical outcomes. This is as seen in the proportion of immunosuppression (CD4 count of less than 500 cells/μL) among HIV-positive persons who reported having been previously diagnosed and on ART (59.2%), compared to 76.0% among those who reported having been previously diagnosed but not on ART (MOH, 2019). The

prevalence of opportunistic infections such as Tuberculosis (54.3%) is also still significantly high in the HIV population. High levels of malnutrition (about 33%) have also been reported among HIV infected adults starting ART in Lusaka, Zambia (Koethe JR, et al, 2010). Malnutrition in the form of macronutrient and micronutrient deficiencies may contribute to the high morbidity and mortality reported among these patients (Stringer JSA, et al, 2006, Heimburger DC, et al, 2010).

1.3 General Objective

To explore the potential role of plasma fat in the augmentation of the immune response and the suppression of the viral load, and how this would correlate with clinical status in Zambian adults with HIV/AIDS

Specific Objectives:

1. To establish the typical baseline and overtime plasma fat status and the corresponding CD4+ and viral load profiles presenting in adult HIV/AIDS patients attending ART clinic at NTH
2. To determine if plasma fat status would predict the absolute CD4+ count and suggest a potential adjunct role of total fat and its sub-types towards immune enhancement in HIV/AIDS patients
3. To determine if plasma fat status would predict HIV infection viral load and suggest an inverse interaction to confirm findings that some plasma fat types have the potential to inactivate HIV in vitro
4. To establish the association between lipid status and the prevalence of opportunistic infections in HIV/AIDS patients
5. To determine if Body Mass Index would predict absolute CD4 count and viral load to suggest its contribution towards immune reconstitution and viral suppression in HIV/AIDS patients

1.4 Research Questions

1. What would be the typical baseline and overtime plasma fat status and the corresponding CD4 and viral load profiles presenting in adult HIV/AIDS patients attending ART clinic at NTH?
2. Would plasma fat status predict CD4+ count and suggest a potential therapeutic role of total fat and its sub-types towards immune enhancement in HIV/AIDS patients?
3. Would plasma fat status predict HIV infection viral load and suggest an inverse interaction to confirm findings that some plasma fat types have the capacity to inactivate HIV in vitro?
4. Is there an association between lipid status and the prevalence of opportunistic infections in HIV/AIDS patients?
5. Would Body Mass Index predict absolute CD4 count and viral load to suggest its contribution towards immune reconstitution and viral suppression in HIV/AIDS patients?

1.5 Hypothesis

Null- Plasma fat status cannot predict CD4+ count or viral load status to suggest a potential role in immune augmentation, viral load suppression and the ultimate improved clinical outcomes in patients with HIV/AIDS

Alternative- Plasma fat status can predict CD4+ count and viral load and has a potential role in immune augmentation, viral load suppression and improved clinical outcomes in patients with HIV/AIDS

1.6 Significance of the Study

There are no investigations conducted before or underway focused on establishing the potential role of macronutrients such as fat in augmenting the immune response and inactivating HIV in vivo in patients with HIV/AIDS in a resource limited setting like Zambia.

There is evidence from prior research suggesting the existence of both micro and macronutrient derangements among the HIV/AIDS population (Drain PK, et al, 2007, Zambia_Nutrition_HIV guidelines, 2011, Visser ME, et al, 2017, Khatri S, et al, 2020, Wang Y, et al, 2021). There is further evidence to suggest that the detection and correction for nutritional imbalances among the HIV/AIDS and non-HIV populations may improve clinical outcomes (Kabagambe EK, et al, 2016, Licona NA, et al, 2016, Njoroge A, et al, 2017, Zyambo K, et al, 2022).

The high HIV prevalence in Zambia (MOH, 2019, USAID, 2022) associated with a high burden of malnutrition among the HIV/AIDS patients (Koethe J R, et al, 2010) should necessitate more research for evidence based patient care. It is therefore anticipated that our study findings may reveal new evidence of the potential role Plasma Fat might play in the comprehensive management of our HIV/AIDS patients. Findings may further inform and justify the need for policies and protocols designed to promote timely identification of nutritional imbalances such as dyslipidaemia and their management through appropriate interventions to improve clinical outcomes for people living with HIV/AIDS.

1.7 Study Limitations and Delimitations

Limitations: In this prospective cross sectional study no causal inferences were expected for deduction (Taris TW, et al, 2021). The study was however expected to reveal associations and the direction of such established associations involving the independent and dependent variables.

The potential for measurement error may have arisen during the testing process of the biomarkers of interest namely fat, CD4 count and viral load and for the physical parameters such as Body Mass Index. Measurement error may have the potential to affect the quality of measurements and the reliability of statistical analyses (Weber P, 2017)

Delimitations: The target population of interest being adult HIV and AIDS patients who were recruited from one district, may have limited the extent to which our study findings could be applied to the general population. It is argued though that initial studies should focus on small populations and have high internal validity, until causal mechanisms have been proven and then the intervention can be scaled up to larger studies with more diverse populations and settings and greater external validity (Burchett HED, et al, 2020)

The study did not consider patients with HIV/AIDS and presenting with comorbidities or conditions that could significantly compromise the body's immunity and restrain the potential for immune reconstitution (Lorenc A, et al, 2014).

The study mainly focused on the role of the major plasma fat subtypes namely cholesterol and triglycerides in HIV disease progression.

1.8 Study Context

Malnutrition is a worldwide problem affecting billions of people. The geographic overlap of high HIV prevalence, malnutrition, and chronic food insecurity in much of sub-Saharan Africa has highlighted the need for more comprehensive approaches to health care (Koethe J R, et al, 2009)

In Zambia, a country with an adult HIV prevalence of 12%, a high burden of malnutrition has been observed among HIV infected adults presenting to the ART clinics. In one review of HIV infected adults starting ART in Lusaka, Zambia, about 33% of patients were found to be undernourished by WHO criteria (BMI < 18.5 kg/m2) and 9% were severely malnourished (BMI < 16kg/m2) (Koethe J R, et al, 2010).

The interaction between HIV/AIDS and malnutrition is inter-dependent and can have a devastating impact on clinical outcomes and the socio-economic status of the affected. This motivated the need to under-take this study seeking to examine the potential role that nutrients such as fats could play in improving clinical outcomes in people with HIV/AIDS.

Most prior research has demonstrated a potential role of fats in the management of several diseases especially of cardiovascular origin (Carrol D N and Roth M T, 2003, L. Djouss´e, S. C, et al, 2003), and relatively few focused on HIV related clinical outcomes. Together with other nutrients found to be beneficial in previous studies understanding the role of fats may impact positively in the comprehensive care for people living with HIV/AIDS in Zambia and worldwide.

Chapter Two: Literature Review

2.0 Introduction

This chapter presents results of literature review. According to Kombo and Tromp (2014, p62), a literature review is, “an account of what has been published on a topic by accredited scholars and researchers”. “It permits the researcher to investigate different theories relevant to the research topic. It allows the researcher to identify the gap in knowledge. In this study, reviewing literature from various sources will enable the researcher to get a deeper understanding of the potential role fats might play in improving clinical outcomes and also identify the knowledge gap that may exist. This forms the basis of the research and more precisely the study objectives.

2.1 Literature Review

Global Perspective

There are approximately 38.4 million people currently living with HIV globally and tens of millions of people have died of AIDS related causes since the beginning of the epidemic (UNAIDS, 2022). Approximately two thirds of these cases are in Sub-Saharan Africa (UNAIDS, 2017). HIV not only affects the health of individuals, it impacts households, communities and the development and economic growth of nations (Global HIV/AIDS Epidemic, 2017). Many of the countries hardest hit by HIV also suffer from other infectious diseases, food insecurity and other problems.

Despite the challenges posed by the HIV epidemic there have been various ongoing interventions to mitigate the impact of the disease. As a result the number of people newly infected with HIV receiving treatment increased to more than 19 million in 2016 (UNAIDS, 2017). However, recent data shows that the pace of decline in new infections is too slow to reach global targets (UNAIDS, 2017). Further global efforts are now advising a more comprehensive management approach than administration of ART alone as being more appropriate to achieve optimal clinical outcomes.

Therefore, the need to explore all the possible determinants of disease progression and their potential role in the comprehensive health care package.

The Global Nutrition Report further shows that 44% of countries with data available (57 out of 129 countries), now experience very serious levels of both under nutrition and, adult overweight and obesity. Despite good progress in some countries, the world is off track to reduce and reverse the trend. Malnutrition which is on the rise worldwide is one such determinant of disease progression (Global Nutrition Report, 2016). In the setting of the HIV/AIDS population malnutrition has been shown to worsen the effects of HIV by weakening the immune system and HIV also in turn promoting nutritional deficiencies (FAO, 2002)

A number of studies conducted globally have demonstrated the important role fat plays in health when consumed within the recommended dietary allowance. Total cholesterol could be used as important biomarker since lipids have a role in viral entry, uncoating, replication, protein synthesis, assembly, budding and infectivity (Lorizate M and Krausslich H, 2011, Mazzon M and Mercer J, 2014, Adal M et al, 2018). Other studies suggest that cells of the immune system in individuals with hypercholesterolemia had greater phagocytic activity, more circulating lymphocytes, more total T cells, more CD8+ T cells, more immunoglobulin production, more proliferation and differentiation, and migration of lymphocytes than from individuals with lower cholesterol levels (Muldoon MF, et al, 1997, Hannedouche S, et al, 2011, Liu C, et al, 2011)

Polyunsaturated fatty acids on the other hand are considered protective against degenerative pathologies in a balanced ratio of n-6: n-3 close to 1. A high ratio is associated with promoting pathogenesis of many diseases such as; cardiovascular diseases, cancer, diabetes, obesity and metabolic syndrome related disease (Candela, C, et al, 2011).Therefore, appropriate amounts of dietary n-6 and n-3 fatty acids need to be considered in making dietary choices and recommendations. N-3 fatty acids have been known to modulate biomarkers such as C-reactive protein (CRP) and CD4 count which are important determinants in the progression of HIV disease and other inflammatory conditions (Okamoto Y, et al, 2009, Reinders I, 2012), and therefore have the potential to improve clinical outcomes in HIV patients.

A case control study conducted in China, examined lipids profile among ART-naïve HIV infected patients and men who have sex with men (MSM) in China. The study compared lipids parameters such as triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and atherogenic index of plasma (AIP) between MSM HIV-infected patients and MSM HIV negative controls. The study conclusions were that HIV infection contributed to decreased total Cholesterol, LDL-C and HDL-C. Further, the acute Human Immunodeficiency Virus infection contributed to higher atherogenic index of plasma, which findings indicated the need for earlier HIV diagnosis and better prevention of dyslipidemia in China (Wang Qi, et al, 2016)

Regional Perspective

Africa accounts for the highest burden of HIV/AIDS and malnutrition thereby posing a major challenge to efforts directed at combating the HIV/AIDS epidemic. Sub-Saharan Africa in particular has the highest prevalence estimates of undernourishment in the world with 23.2% of its population affected (FAO, 2015). The region also has the highest burden of HIV infection constituting 69% of the estimated 36.7 million people living with HIV globally in 2015 (UNAIDS, 2016). In the face of the high global HIV/AIDS disease burden, there has been more than a two fold increase in the number of HIV positive people receiving ART in Eastern and Southern Africa, the world's most affected regions (Takarinda K C, et al, 2017).

The scale-up of ART has resulted in AIDS related deaths in the region decreasing by 36% since 2010. However, there are still factors suggested to be associated with poor outcomes. In Sub-Saharan Africa, a high prevalence of malnutrition has been reported among people living with HIV/AIDS (Nanewortor et al, 2021, Molla M, et al, 2022). Malnutrition in the form of low body mass index is common at ART initiation ranging from 10% to 33%. (Uthman O A, 2008, Weiser SD et al, 2009). Prior studies done in Zambia reflect similar findings.

A study conducted in Uganda by Kabagambe EK, et al, 2016, examined the association between plasma n-6 fatty acid levels and CD4+ count, hospitalization and mortality in HIV infected patients. The findings showed that higher levels of gamma-linolenic acid, dihomo-gamma linolenic acid, arachidonic acid and aolrenic acid were associated with higher CD4 counts at baseline and that higher plasma levels of gamma-linolenic acid were independently associated with reduced risk of death or hospitalization in HIV/AIDS patients. These results further suggested a potential for using n-6 fatty acids to improve outcomes from antiretroviral therapy.

In a Kenya based nested cross sectional study, non-fasting total Cholesterol (TC) and High Density Lipoprotein Cholesterol (HDL) levels were measured by standard lipid spectrophotometry on thawed plasma samples obtained from HIV-infected participants and their uninfected partners. Dyslipidemia, defined by high TC (>200 mg/dl) or low HDL (<40 mg/dl) was compared between HIV-infected and uninfected men and women.

The study findings revealed a high prevalence of dyslipidemia characterized by low-HDL and associated with a high viral load and low CD4 cell count. This suggested that screening for dyslipidemia in ART naïve individuals, even in a non-fasting state, is still important as it would identify HIV-infected individuals at elevated risk of developing an even higher TC: HDL ratio upon ART initiation and may help inform the choice of their ART drug regimen (Njoroge A, et al, 2017). Similar findings have been suggested in a cross sectional study performed in Ethiopia. In this study, a high prevalence of dyslipidemia among HIV infected patients receiving first line ART was reported. Study findings suggested a need for monitoring of lipid levels in patients with HIV on long term first-line ART with a special attention to be focused on order age, urban residence, longer duration of ART use, high BMI and smokers (Fiseha T, et al, 2021)

Local perspective

Research conducted in 2007 by the Centres for Infectious Disease Research in Zambia (CIDRZ), the World Food Programme (WFP), and the U.S. Centers for Disease Control and Prevention (CDC) found that malnutrition among people living with HIV (PLHIV) was much higher than in the general population. Of adults starting antiretroviral drugs (ARVs), 33.5% were moderately malnourished, with a Body Mass Index between 16.0 kg/m2 and 18.5kg/m2, and 13.5% were severely malnourished, with a BMI < 16 kg/m2. (Lusaka District Health Management Team [LUDHMT] and CIDRZ 2007) in (Zambia_Nutrition_HIV guidelines, 2011).

Malnutrition among the HIV/AIDS patients in Zambia can present in the form of micronutrient and macronutrient deficiencies. Common micronutrient deficiencies include Vitamins A, C, E, B6, B12, Zinc, Iron and Selenium while macronutrient deficiencies may involve depletion of nutrients such as carbohydrates, protein, fibre and fat (Zambia_Nutrition_HIV guidelines, 2011:10-11)

The role of vitamins and mineral supplements in malnourished African HIV-infected adults starting antiretroviral therapy was examined in a clinical trial conducted between August 2011 and December 2013 at the UTH, Lusaka, Zambia and the National Institute for Medical Research (NIMR) in Mwanza, Tanzania. The study called the Nutritional Support for African Adults Starting Antiretroviral Therapy (NUSTART) trial was designed to test if a high content of vitamins and minerals in a lipid-based nutritional supplement (LNS) would decrease mortality of malnourished adults referred for ART (Filteau S, et al, 2015). The results suggested that changes in serum electrolytes, largely irrespective of the starting point and the direction of change, were more strongly associated with mortality than were absolute electrolyte levels. The research recommended that, although K and phosphate are required for tissue deposition during recovery from malnutrition, further studies are needed to determine whether specific supplements exacerbate physiologically adverse shifts in electrolyte levels during nutritional rehabilitation of ill malnourished HIV patients (Rehman AM, et al, 2017)..

2.2 Critical review of Empirical Studies

HIV Infection and the immune system: Both CD4+ and CD8+ T cells are important in controlling HIV infection. HIV infection stimulates production of cytokines such as TNF*α*, IL-6, IL-10, and IFN*γ* and a pool of activated target cells in the lymphoid tissue which paradoxically help in establishing and propagating HIV infection (Duggal S, et al, 2011). Similarly, some studies have suggested alternative immune mechanisms suggesting that immune activation (IA) in the natural history of HIV infection covers a broad spectrum of cellular processes and that untreated HIV-infected patients display elevated markers of activation in most cell compartments, especially expression of the surface markers CD38 and HLADR on T cells (Liu Z, et al 1998, Hazenberg M D, et al, 2003). This entailing therefore that immune activation in HIV infection should be understood from a broader context than focusing on the role of CD4+ count in isolation. In this vein, the role of fats in the immune mechanisms goes beyond its interaction with the CD4 T cells or the virus alone even though these will be the main focus for the study. Further, studies have shown that during viral infection in vivo, the innate immune system produces interferons (IFNs) that are involved in up-regulation of interferon-stimulated genes (ISGs) such as Cholesterol-25-hydroxylase (Ch25h) that can convert cholesterol to 25-hydroxycholesterol (25-HC). 25-HC inhibits viral entry by blocking membrane fusion between virus and cell (Homes R, et al, 2011, Liu S, et al, 2013). In contrast, Klein SL, (2000) suggests that Males are more susceptible than females to infections due to the differences in endocrine-immune interactions and to favor this, studies have shown that plasma HIV-1 RNA levels in women are lower than in men (Sterling TR, et al, 2001, Ghandi M, et al, 2002) and that treatment with estrogen protects against the transmission of simian immunodeficiency virus (SIV) (Hel Z, et al, 2010).

Regarding malnutrition and the immune system, Enwonwu C O, (2006), makes the case that malnutrition elicits dysfunctions in the immune system and promotes increased vulnerability of the host to infections. These immune dysfunctions are referred to as nutritional-acquired immune deficiency syndrome (NAIDS) and every type of immunological deficiency induced by malnutrition can be included under the NAIDS umbrella (Duggal S, et al, 2011).

Some of the notable nutrient deficiencies that can adversely affect the immune mechanisms include: deficiencies of essential amino acids which can depress the

synthesis of proteins responsible for production of cytokines released by lymphocytes, macrophages, and other body cells, complement proteins, kinins, clotting factors, and tissue enzymes activated during acute phase responses (Beisel W R, et al, 1996). Arginine deficiency diminishes the production of nitric oxide, and hence, the antioxidants, allowing damaging effects of free oxygen radicals ((Beisel W R, et al, 1996).

The “nonessential” amino acid glutamine is necessary for lymphocytes and other rapidly growing cells. The essential fatty acids, particularly the n-3 fatty acids, serve as the key precursors for the production of eicosanoids like prostaglandins, prostacyclins, thromboxanes, and leukotrines that play a variety of host defensive roles. Thus their deficiency in the diet can impair cytokine synthesis (Chavali S and Forse R A, 1994).

Similarly, an in vitro study demonstrated that beta-estradiol inhibited HIV-1 replication in human peripheral blood lymphocytes(Zhang M, et al 2008, Adal M et al, 2018) by inhibiting target cell infection that involves cell-entry through higher expression of chemokines (Rodriguez-Garcia M, et al, 2013). In converse, Li X, et al, (2019) suggest that Body Mass Index plays a role in immune mechanisms among the HIV-infected individuals on antiretroviral therapy. They report from their longitudinal cohort study that higher baseline BMI could predict better immune reconstitution in HIV-infected patients after HAART initiation. Similar reports have suggested that, the immune reconstitution induced by HAART is often greatest among subjects classified as overweight (Koethe JR, et al, 2011, Johnson KD, et al, 2014),and there is some evidence that a higher BMI is associated with more robust CD4+ T-cell recovery in HAART-treated patients (Koethe JR, et al, 2016). However, in the study by Li X, et al, (2019) some notable limitations were that pharmacokinetic data was not collected, thus, it remained unknown whether the treatment (e.g. drug dosing) of overweight/obese patients could result in drug exposure as compared to normal weight patients. In addition, this observational study might not provide evidence of a direct causal pathway between BMI and immunologic reconstitution. On the contrary Tedaldi EM, et al, (2006) report in their study findings that increased BMI was not associated with decreased virologic and immunologic responses to initial HAART and that the responses were equivalent and within expected ranges among normal weight, overweight and obese patients at 3 to 9 months of observation. These inconsistent revelations on the effects of BMI on immune reconstitution following antiretroviral therapy initiation do underscore the need for further exploration.

Regarding the bioavailability of drugs, lipids are used as carriers for poorly water soluble drugs (Pouton C, 2006, Sachin Aryal et al, 2020). Due to the ability of lipids to improve the solubility and bioavailability of poorly water soluble drugs, the lipid based drug delivery system has gained much interest in recent years (Jannin V, et al, 2008). In this

case a study has revealed that hypocholesterolemia impairs HAART effectiveness in HIV infected patients (Miguez MJ, et al, 2010).

Theory about HIV induced inflammation

HIV infection has been associated with chronic inflammation as suggested by evidence of elevated levels of interleukin-1-beta (IL-1β), interleukin-6 (IL-6), gamma IFN (IFN-γ), and tumor necrosis factor-alpha (TNF-α) detected in the serum, cerebrospinal fluid, and cell culture supernatants of HIV-infected persons (Hunt PW, 2012). Alternatively, indirect mechanisms, such as activation of immune cells by HIV gene products, suboptimal immune control of chronic viral infections (e.g., CMV, Epstein-Barr virus), and HIV-induced disruption of the gastrointestinal mucosa with subsequent bacterial translocation into the systemic circulation are known to contribute to chronic immune activation and release of proinflammatory cytokines (Appay V and Sauce D, 2008). On the other hand some studies have shown that the pathogenesis of HIV enteropathy is the result of both direct and indirect effects of the virus - gp120 which negatively affects tubulin depolymerization, and induction of local cytokines such as interleukin (IL)-6, IL-10, tumor necrosis factor causing altered epithelial ionic balances and enterocyte apoptosis (Schmitz H, et al, 2002, Maresca M, et al, 2003, Kotler DP, 2005, Crum-Cianflone NF, 2010), and that the associated inflammation, increased permeability, and malabsorption (of bile acids and vitamin B12) all contribute to the diarrhea that occur with HIV enteropathy. Regarding CD4+ T cell loses in the gut of the HIV infected some researchers have argued that, while profound loss of CD4+ T cells in gut tissues is a salient event of the HIV-1 disease course, it is important to point out that these events alone cannot fully explain progression to AIDS or the broad dysfunction that exists in the GI tract (Mudd JC and Brenchley JM, 2016). They further report that uninfected humans with idiopathic CD4+ lymphopenia exhibit very low numbers of CD4+ T cells in the GI tract without any evidence of systemic inflammation (Kovacs SB, et al, 2015), indicating that there are other pathological phenomena that must contribute to HIV-1pathogenesis. Regarding therapeutics, a clinical trial has shown that compared to cART alone, IL7 therapy achieved more sustained CD4+ T-cell restoration.in the majority of participants (Thiebaut R, et al, 2016), and that taken together, the novel therapeutic strategies outlined above may promote effective immune reconstitution by restoring structural and immunological components of the GI tract (Mudd JC and Brenchley JM, 2016)..

Theory about HIV disease progression and mortality associated with HIV related inflammation and immunodeficiency

In this theory it is suggested that proinflammatory cytokines (e.g., IL-1β, TNF-α) have been shown to up regulate HIV replication in cells at the entry and transcription steps of the viral lifecycle and induce apoptosis of activated CD4+ T lymphocytes ((Appay V and Sauce D, 2008). Further evidence reveals that proinflammatory cytokines have thymosuppressive effects and may reduce the regenerative capacity of the thymus to replace lost immune cells (Sempowski GD, 2000).

When this happens, latent co-pathogens, such as CMV and herpes zoster, can reactivate and further contribute to the cycle of immune activation, inflammation, and immunodeficiency ((Appay V and Sauce D, 2008)

Theory about the regulation of inflammation and immunity by fatty acids

A large body of literature has demonstrated that EPA and DHA reduce plasma concentrations of inflammatory cytokines (Fetterman JW, et al 2009). One mechanism suggested is that when consumed as fish or fish oil supplements, EPA and DHA replace arachidonic acid in cell membranes and inhibit the synthesis of proinflammatory arachidonic acid metabolites (Maroon JC, et al, 2006). Further, in clinical trials, fish oil supplementation has been associated with symptom relief and reductions in serum levels of proinflammatory cytokines in persons with rheumatoid arthritis and asthma, (Adam O, et al, 2003 and Michaelborough TD et al, 2006).

Similarly, in a systematic review and meta-analysis it has been confirmed that early intervention with Omega -3 fatty acid emulsion in gastrointestinal cancer can not only improve the postoperative indicators of immune function, reduce inflammatory reaction, and improve the postoperative curative effect but also improve the immune suppression induced by conventional parenteral nutrition (PN) or tumor. The study further suggests that postoperative patients with gastrointestinal cancer should add omega-3 unsaturated fatty acids in their PN formula and that further high-quality RCTs are needed to verify its efficacy (Zhao Y & Wang C, 2018).

Comparable findings are suggested in a clinical trial were n-3 PUFA supplementation was associated with decreasing viral load trend in the beneficiaries and an increase in the control group (Licona NA, et al, 2016). This relates with the understanding that fatty acids inactivate animal enveloped viruses such as myxoviruses, paramyxoviruses, arboviruses, and herpes viruses within minutes of contact at a concentration of 5–25 μg/ml (Kohn A, et al, 1980) and it has been considered that DHA may also posses similar capacity to inactivate HIV (Das UN, 2005). Other studies as reported in (Kabagambe EK, et al, 2016) suggest similar findings: Polyunsaturated fatty acids (PUFA) improve humoral immune responses (Ramon S, et al, 2014) and survival in mice (Svahn SL, et al, 2015). In vitro, n-6 fatty acids such as arachidonic acid confer resistance to infection by both bacteria and viruses (Chouinard F, et al, 2013). These benefits have also been observed in vivo. In macaque monkeys arachidonic acid is

associated with an increase in the T-helper 17 (TH17) CD4+ cell compartment, (Filteau S, et al, 2015) which is known to lower viral loads for the simian immunodeficiency virus (Hartigan-O'Connor DJ, et al, 2012).

On the contrary findings in one study have revealed that no significant changes in interleukin-6 (IL6), interleukin-1 beta (IL1-beta) and tumor necrosis factor-alpha (TNF-alpha) serum concentrations were observed with fish oil supplements for 12 weeks. In this study the objective was to assess the effects of a low dose of marine omega-3 fatty acids on inflammatory marker concentrations in HIV-infected subjects under antiretroviral therapy (ART) (Oliveira JM, et al, 2015).

Focusing on nutritional status as a determinant a study has revealed that a low baseline BMI and an attenuated CD4+ cell response at 6 months had a compounding, negative impact on post-6 month survival (Koethe J R, et al, 2010). In a study from Cote d'Ivoire comparing patients with a BMI above and below 18.5 kg/m2, no difference was found in the proportion who failed to gain at least 50 cells/μL at 6 months following ART initiation (Toure S, et al, 2008). However, in a study from South Africa, a BMI in the lowest quartile (<17.1 kg/m2) was associated with a failure to achieve a CD4+ count ≥200 cells/μL at 12 months (Barth RE, et al, 2008).

Evidence from a number of studies has further shown that HIV infection triggers pronounced oxidative stress in both laboratory models and the context of *in vivo* infection.

In this vein HIV-infected individuals exhibit enhanced ROS production in monocytes (Elbim C, et al, 1999) and severely elevated levels of oxidized nucleic bases such as 8-oxoG and lipid peroxidation products, including MDA in plasma and alkanes in the breath output (Sonnerborg A, et al, 1988, Wanchu A, et al 2009). Working through their anti-oxidant and anti-inflammatory properties fatty acids could have counter-regulatory effects against such pathogenetic mechanisms in HIV infection.

2.3 Knowledge gap

Most prior research has quite extensively examined the role of micronutrients in HIV/AIDS disease progression (Ferencik M, et al, 2003, Drain PK, et al, 2007, Visser ME, et al, 2017, Khatri S, et al, 2020, Wang Y, et al, 2021). Data suggesting the potential role of macronutrients such as fats in HIV/AIDS disease progression with regard to the immunological and virological status is lacking.

Most studies involving fats such as cholesterol and triglyceride have examined their role in the management of non-communicable disease such as cardiovascular disease, diabetes and obesity (Carrol D N and Roth M T, 2003, L. Djouss´e, S. C, et al, 2003, Candela, C, et al, 2011, Banik S and Hossain MS, 2014).

The role of fats in the management of HIV/AIDS in which acute and chronic inflammation and oxidative stress is characteristic has not been adequately examined. Similarly, most studies involving fatty acids have explored their role in the management of inflammatory diseases such as Rheumatoid arthritis, Allergies, Chronic Obstructive Pulmonary Disease, Psoriasis, cardiovascular and neuro-degenerative disease rather than that in the management of HIV/AIDS.

Chapter Three: Theoretical and Conceptual Framework

3.0 Theoretical Framework

The theoretical framework is a summary of the researchers theory regarding a particular problem that is developed through a review of previous research on the variables involved (Nsenduluka E, 2017). A theoretical framework will be applied in this research to provide a rationale for predictions about the relationships among variables of the study. In this study, the key variables will include total cholesterol, LDL-c, HDL-c, triglyceride CD4 T cell count and HIV viral load.

The rationale of the study is based on the observation that despite the introduction of cART, high rates of morbidity and mortality are still reported among some HIV patients due to their failure to achieve full immune reconstitution (Gaardbo J C, et al, 2011).

Treatment of HIV infection with combination antiretroviral therapy (cART) usually results in diminished viral replication and increasing CD4+ cell counts. However, approximately 20% of all HIV-infected individuals fail to restore their CD4+ cell counts despite optimal treatment and fully suppressed viral replication (Autran B, et al, 1999, Piketty C, et al 1998). Based on the literature review citations in chapter 2, the following among the theories for consideration highlighted how fats may interact with HIV and the immune system and impact upon clinical outcomes; (i) the HIV induced inflammation, a work of proinflammatory cytokines, (ii) the cycle of immune activation, inflammation and immunodeficiency preceeds HIV disease progression and mortality and, (iii) inflammation and immune regulation, the work of the pro and anti-inflammatory fatty acids.

3.1 Conceptual Framework

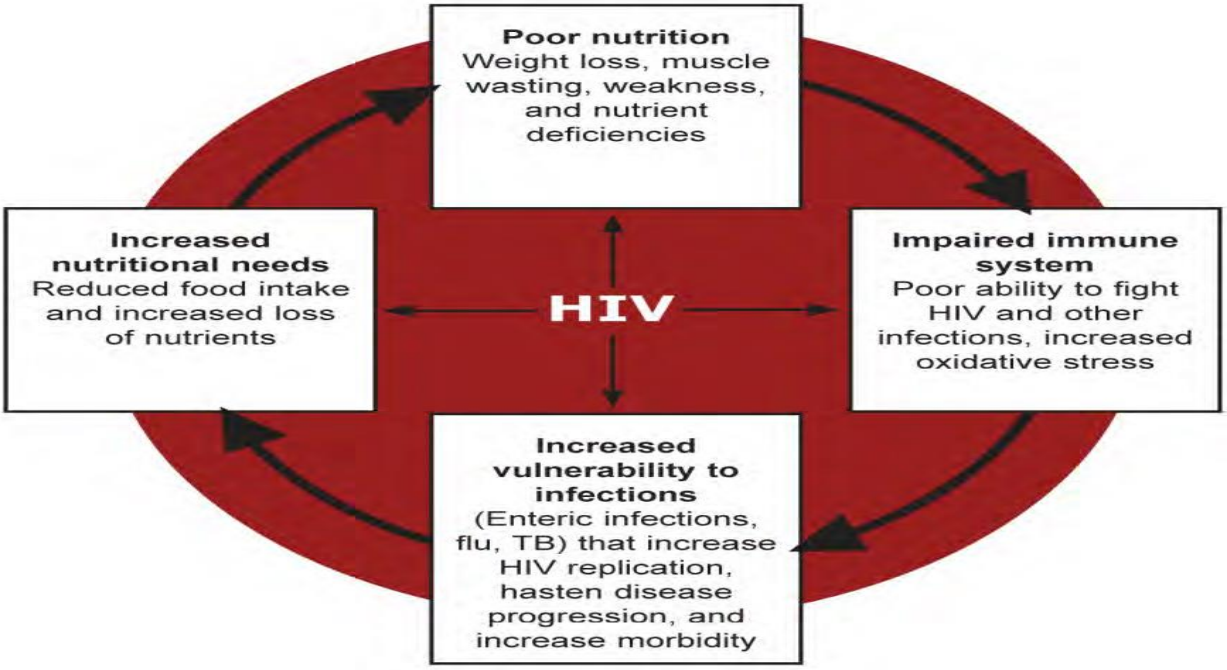
A conceptual framework helps the researcher articulate their thinking and explains the relationship among interrelated constructs and the probable connection in the study constructs (Nsenduluka E, 2017).

In this study, the conceptual framework was developed based on the understanding that malnutrition and HIV relate in a vicious cycle.

Malnutrition weakens the immune system, which worsens the effects of HIV, which then increases the likelihood of malnutrition. People with HIV have an increased risk of malnutrition because of reduced food intake, reduced nutrient absorption and reduced nutrient utilization (Zambia_Nutrition_HIV guidelines, 2011:8).

This then results in deficiencies in micronutrients and macronutrients including fats all of which are known to support the immune system. Figure.3.2, demonstrates the relationship.

Figure 3.2. Cycle of poor nutrition and infection in a person with HIV



Source: Food and Agriculture Organization of the United Nations (FAO). 2002. *Living Well with HIV/AIDS: A Manual on Nutritional Care and Support for People Living with HIV/AIDS*. Rome.

Based on the relationship in figure 3.2, the following framework (figure 3.3a) is specifically designed to demonstrate how fats, in the course of ARVs and the prevention and treatment of opportunistic conditions can interact to enhance CD4+ counts and suppress viral load leading to good clinical outcomes. The framework is further elaborated in (figure 3.3b).

Figure 3.3a. A framework depicting the potential role of fat in the comprehensive management of HIV/AIDS

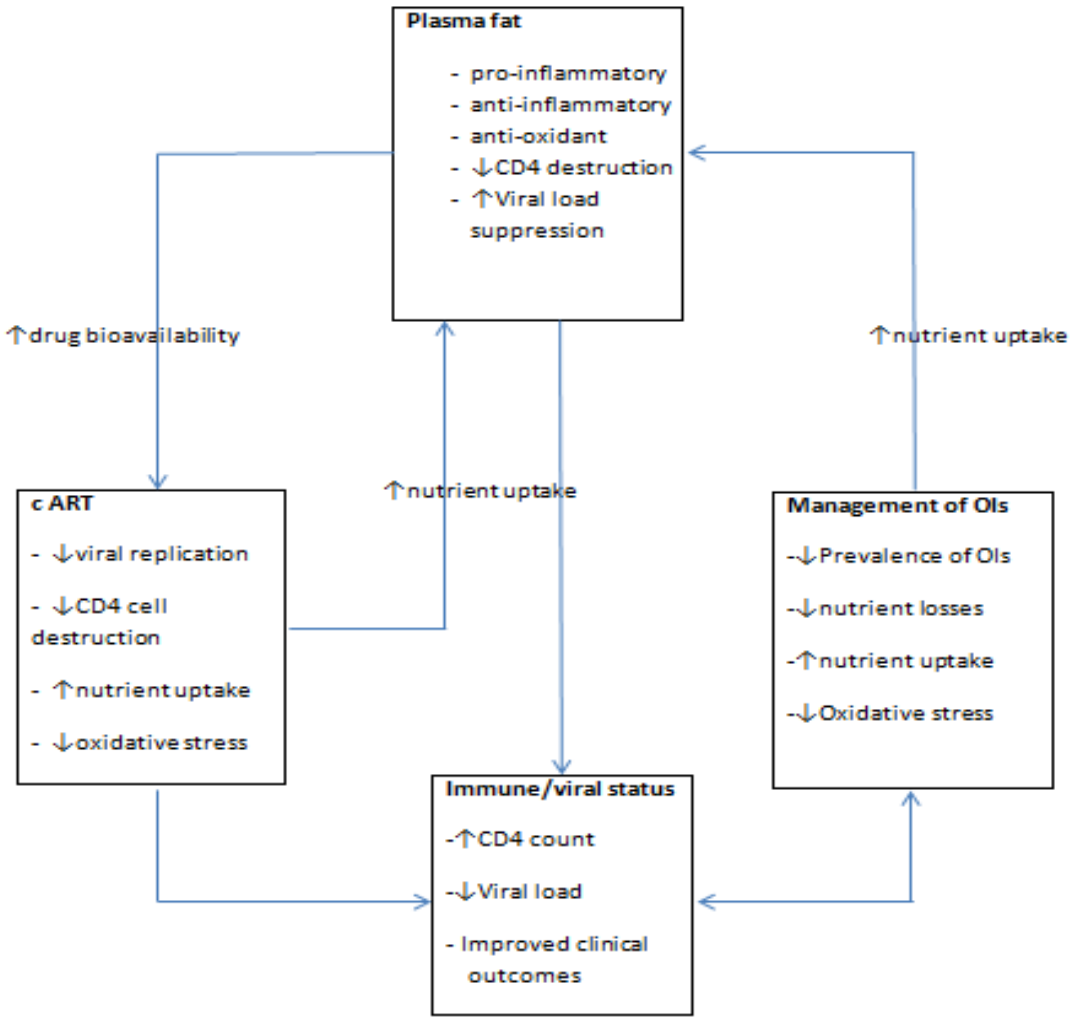
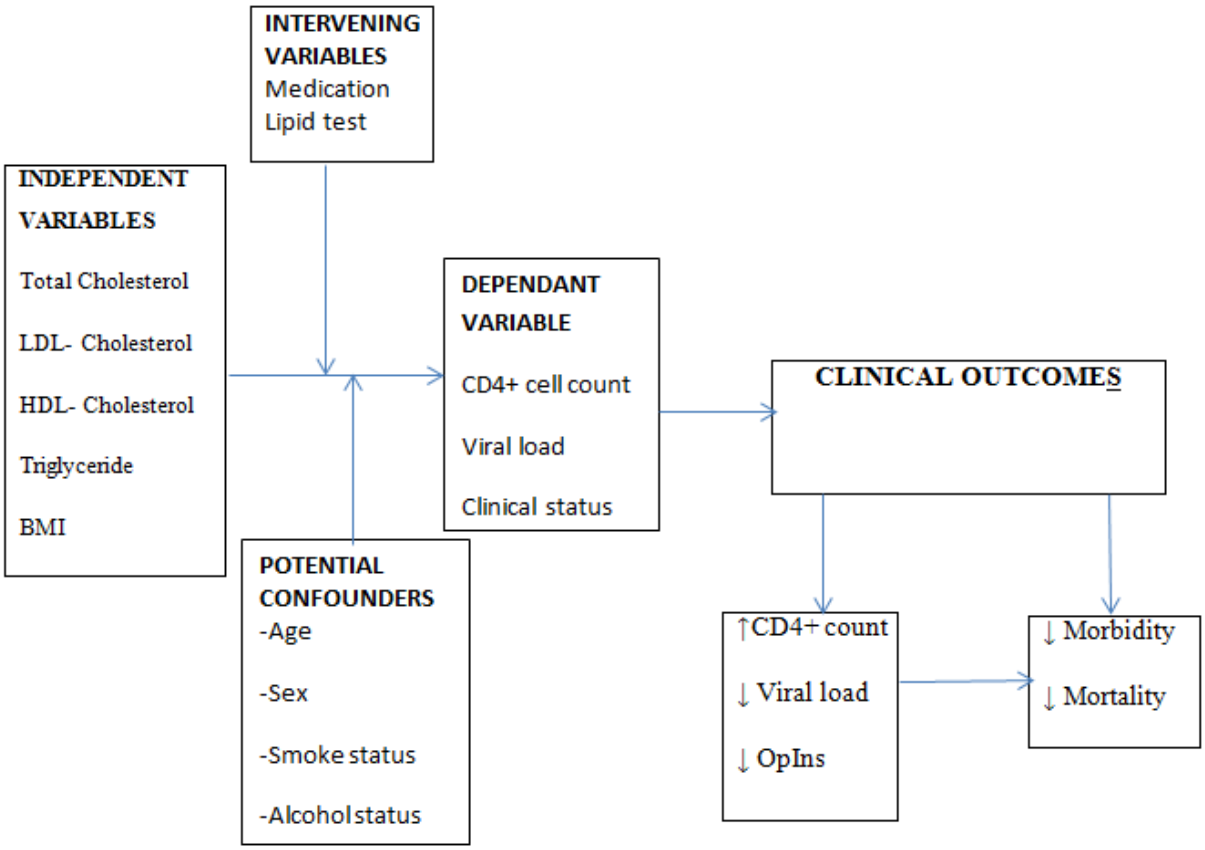


Figure 3.3b. Conceptual framework depicting the interaction between the independent and dependent variables with potential confounding



3.2 Operationalization of Concepts

The key variables for the study will be measured and reported as follows:

Total Cholesterol: A proportion of total Plasma Fat to be measured in mmol/l

LDL-C: A proportion of total Plasma Cholesterol to be measured in mmol/l

HDL-C: A proportion of total Plasma Cholesterol to be measured in mmol/ l

Triglyceride: A proportion of total Plasma Fat to be measured in mmol/l

Absolute CD4+ count: A measure of immune status to be expressed as the number of cells per microliter of blood

Plasma Viral Load: A measure of the amount of HIV in the blood to be reported as number of copies per mililitre

3.3 Summary

With approximately 38.4 million people currently living with HIV and tens of millions dying of AIDS related causes worldwide (UNAIDS, 2022), the HIV/AIDS epidemic continues to impact negatively upon households, communities and the development and economic growth of nations (Global HIV/AIDS Epidemic, 2017). Although the number of people newly infected with HIV, receiving treatment increased to more than 19 million in 2016 (UNAIDS, 2007), recent data shows that the pace of decline in new infections is too slow to reach global targets (UNAIDS, 2017). As such, global efforts are now advising a more comprehensive management approach than relying on the role of ART alone to achieve optimal clinical outcomes.

Malnutrition which is on the rise worldwide is an important determinant of disease progression (Global Nutrition Report, 2016), whose role in the comprehensive health care package for HIV/AIDS deserves exploration.

Africa accounts for the highest burden of HIV/AIDS and malnutrition thereby posing a major challenge to efforts directed at combating the HIV/AIDS epidemic.

Sub-Saharan Africa in particular has the highest prevalence estimates of undernourishment in the world with 23.2% of its population affected (FAO, 2015). The region also has the highest burden of HIV infection constituting 69% of the estimated 36.7 million people living with HIV globally in 2015 (UNAIDS, 2016).

In Zambia, malnutrition among the HIV/AIDS patients presents in the form of micronutrient and macronutrient deficiencies. Common micronutrient deficiencies include Vitamins A, C, E, B6, B12, Zinc, Iron and Selenium while macronutrient deficiencies may involve depletion of nutrients such as carbohydrates, protein, fibre and fat (Zambia_Nutrition_HIV guidelines, 2011:10-11)

Most previous studies have quite extensively examined the role of micronutrients in HIV/AIDS disease progression. Data suggesting the potential role of macronutrients such as fats in augmenting the immune response and suppressing viral load in HIV/AIDS patients in resource limited settings like Zambia is lacking. The study findings therefore, are expected to add to the body of knowledge suggesting the role of fat in improving clinical outcomes as determined by its contribution to immune mechanisms and viral suppression.

Chapter Four: Research Design and Methods

4.0 Introduction:

According to Kombo and Tromp (2014, p. 70), research design is the “glue” that holds all of the elements in a research project together. A design is used to structure the research, to show how all of the major parts of the research project work together to try and address the central research questions. In selecting a research design for this study, the researcher considered the conditions of subjects, time and resources available for the study. The design in this case was cross sectional and quantitative. Research methods on the other hand are the strategies, processes or techniques utilized in the collection of data or evidence for analysis in order to uncover new information or create better understanding of a topic (UoN, 2022). The methods are as detailed in section 4.1.

Philosophical Underpinning

This was a quantitative research which involved the collection and analysis of numerical data to describe characteristics, deduce predictions and the testing of hypotheses. Therefore Positivism would be the most appropriate philosophical ideology. Positivism, in Western philosophy, is generally, any system that confines itself to the data of experience and excludes a priori or metaphysical speculations. Its basic affirmations are that all knowledge regarding matters of fact is based on the “positive” data of experience and that beyond the realm of fact is that of pure logic and pure mathematics (Feigl H, 2022).

4.1 Research Methods

In this cross sectional study design, quantitative methods were applied to determine the relationship between the independent and outcome variables with the primary goal being to analyze and represent the interaction through statistical analyses. The study period being from December 2017 to date and the study site being the NdolaTeaching Hospital HIV clinic in Ndola, Zambia. The target population were adult HIV/AIDS patients receiving care from the HIV clinic, who were eligible and consented to participate in the study. A total of 174 participants were recruited for the study. The recruitment process involved providing full information and informed consent

administered by the study staff following which the consenting subjects were enrolled accordingly. Participants were recruited anytime within 12 months of initiating ART.

Following recruitment the study participants were subjected to history taking, physical examination with vital signs and anthropometric measurements at baseline which point they were also screened for opportunistic infections and subjected to the routine and study specific laboratory tests. At 2 weeks following enrolment, participants were further followed up for review of lab work ups and monitoring for drug toxicities in those who would have been recently initiated on cART or prophylaxis medications.

At visit 2 (3 months from baseline) the participants were subjected to clinical assessments and blood sample draws for CD4+ count, viral load and plasma fat testing as at visit 1(figure 4.54).

4.2 Target Population

The target population of the study were Adult HIV/AIDS patients who were men and women aged 18 years and above receiving combination antiretroviral therapy (c ART) from the HIV clinic at Ndola Teaching Hospital (NTH) situated in Ndola district of the Copper belt province of Zambia. The NTH is the second largest tertiary hospital in Zambia with 26 inpatients wards and a bed capacity of 760. It offers specialist referral services to the Copper belt province and the entire northern region of Zambia. At the time of the study the HIV clinic had a total of 4,519 patients retained on the register. Out of the total number registered, 4064 patients were on 1st line cART, 437 on 2nd line and 18 on 3rd line therapy.

Inclusion

1. Adult HIV/AIDS men and women patients aged 18 years and above receiving care from the NTH HIV clinic
2. Participants able and willing to provide informed consent.
3. Participants who intended to remain in the current geographical area for the study at least for the 3 months duration required for a complete follow-up per subject.
4. Participants willing to adhere to a stepped up clinic visit schedule for the 3 months follow-up period and to allow for a possible visitation to their home in the event of missed clinic visits.

Exclusion

The study excluded patients presenting with the following conditions with the potential to confound outcomes or affect adherence to the follow-up clinical schedule

1. Long-term comorbidities such as diabetes, cardiac, renal disease, malignancies such as Kaposi sarcoma, lymphomas and overwhelming infections such as septicemias all with the potential to cause significant immunosuppression.

2. Patients expected to receive a protease inhibitor based ART regimen

3. Patients on a long term immunosuppressive medications

4. Alcohol dependent as determined by the CAGE criteria for alcohol dependency (O'Brien CP, 2008)

4.3 Sample size calculation and justification:

This study involved a population of approximately 174 adult HIV/AIDS patients at baseline. The sample size estimate was derived based on the Cochran formula [$n= z^2pq/d^2$] and its adequacy was further determined by fit testing in a regression model.

In the given formula, n= sample size, p= 13.2% (HIV prevalence), q= 1-p, z= 1.96 and d= 0.05 at 95% confidence interval. This gives an n= 177

Step 1: Based on the Cochran formula we determined the sample size estimate of (n=177) using an HIV prevalence of 13.2% for the Copper belt province (MOH, 2019), and applying a margin of error of 0.05 at 95% confidence interval.

Step 2: Using the multiple linear regression model we expected to fit a model with 174 patient outcomes. We applied the 10 to 15 outcomes per parameter rule in regression modeling strategies (Chowdhury MZI, Turin TC, 2020), to test for sample size adequacy.

Given 174 patient outcomes, the model was able to fit between 11 and 17 parameters. Therefore, with the 12 selected parameters (total cholesterol, LDL-c, HDL-c, triglyceride, CD4+,VL,Clinical Status, Age, Sex, BMI, Smoke and Alcohol Status) the sample size was sufficient for modeling (cite the authority).

4.4 Sampling Procedure

A simple random sampling method was applied to select the eligible study participants attending the HIV clinic at Ndola Teaching Hospital. The technique employed relies on

applying a selection method that provides each participant with the same chance of being selected.

Further, the sampling method gives the study participants an equal opportunity for selection, thereby preventing the potential for bias and making the sample more representative of the study population. In the study, out of the total number of HIV clients on the register, we identified those that were on 1st line therapy as our population of interest. We calculated our expected sample size for the study and then randomly selected the study participants from the population of interest based on the derived sample size. The target group for sampling were clients on 1st line therapy within 12 months of initiating cART. We identified a total of up to 961 patients in this category as they presented for reviews and initiation during the study period. Based on the register of the qualifying subjects we targeted to recruit at least 5 every week by randomly picking any of the patient's id numbers from the list presented on a clinic day. By this approach we projected to achieve enrolling the required sample size of 174 subjects within 8 to 10 months.

4.5 Description of Data Collection Instruments

The Zambian Ministry of Health certified and standardized questionnaires in HIV care were adopted and applied to derive information on history taking, physical assessments and anthropometric measurements. A tape measure and weighing scale were used to determine the height and weight respectively. Needles, syringes, swabs, specimen bottles, strapping and accompanying requisition forms were used for blood sample collection and preparation. The FacsCount machine was used for CD4 count measurement, while the Ampliprep and Taqman machine was used for Viral Load measurement. The Cobas C111 machine was utilized for Plasma Fat analyses.

4.6 Data collection process

4.61 Study protocol

The data collection team comprised a study nurse, a medical officer, laboratory technologist, scientific officer and a supervising physician who were trained and certified in GCP/GCLP.

At the first encounter, the pre-enrollment procedures were explained and informed consent was obtained from the subject, then history taking and physical examination with anthropometric measurements were performed using standardized questionnaires. Blood specimens were taken from each participant to determine plasma fat status, absolute CD4+ counts and plasma viral load at baseline. The routine hematology and biochemistry, CD4+ and viral load samples for the study were received and processed by the study biomedical technologist at the NTH lab, while the sample for plasma fat testing were received and processed for analysis by the study scientific officer based at the TDRC in the Clinical Chemistry and Molecular Biology units

Participants were then followed up at 2 weeks for any necessary clinical or laboratory reviews and 3 months from baseline to repeat the procedures performed at visit 1.

4.7 Standard Operating Procedure

The following steps were followed by the study nurse or clinician to prepare the patient for blood sample draw, its packaging, labeling and submission of specimens for laboratory analyses:

1. Introduce oneself to the patient and confirm patient's identity
2. Explain the procedure to be done.
3. Obtain consent for the procedure
4. Secure privacy
5. Prepare the necessary equipment for the procedure.
    - Sterile syringe
    - Sterile hypodermic needle
    - Antiseptic solution
    - Sterile gloves
    - Cotton wool
    - Specimen bottles for hematology, CD4+ and viral load, renal, liver function and lipid profile analyses)
6. Reassure the patient and place them in a comfortable posture for the procedure

7. Wash and dry your hands, then put on the sterile gloves

8. Clean the area overlying the vessel from which blood is to be drawn, with cotton swab soaked in antiseptic solution.

9. Draw a sample of blood and withdraw the needle (about 4 to 5 mls of blood will be required for each specimen bottle to analyze the parameters of interest for the study

10. Place a dry swab over the area where the needle was pushed and press over it at least for a minute to ensure hemostasis is achieved.

11. Dispose of your gloves and equipment in the clinical waste bin. Wash your hands.

12. Thank the patient.

13. Samples drawn were then placed in a cooler box pending delivery to the respective NTH and TDRC laboratories by a trained potter.

4.8 Data Management

All the 4519 HIV patients under the Ndola Teaching Hospital HIV care and treatment at the time of the study had their medical records captured both electronically and in a secure and confidential hardcopy filing system. The electronic patient information tracking and processing system utilized is the District Health Management Information System (DHIMS).

The data captured from the clinical assessments and laboratory records were entered in excel and then exported to STATA for statistical analyses.

The study involved a qualified and experienced researcher in Medical Sciences who was not directly involved in the project, as a Data Monitoring Officer to monitor progress on the research from its inception to conclusion.

4.90 Data Preparation and Analysis

4.91 Introduction

This section presents the process of data preparation and analysis. Data analysis is a process of systematically applying statistical and /or logical techniques to describe and illustrate, condense and recap, and evaluate data.

There are two kinds of data the researcher is likely to work with namely quantitative and qualitative data. Quantitative data refers to the information that is collected as, or can be translated into, numbers, which can then be displayed and analyzed mathematically while qualitative data are collected as descriptions, anecdotes, opinions, quotes or interpretations (Nsenduluka E, 2017). Quantitative data analysis was applied in this study.

4.92 Data preparation and analysis

The baseline characteristics of participants by gender were compared using Wilcoxon rank sum test for continuous variables and chi-square test for categorical variables.
The main research question was addressed by establishing the association between plasma fat and immunological status and that between plasma fat and virological status adjusted for potential confounders using the multiple linear regression model.

Specific Objective 1

Being continuous variables, we analyzed and compared plasma fat, CD4 and viral load using Wilcoxon rank sum test. We further depicted the change in plasma fat status from visit 1 to visit 2 and the corresponding change in status for CD4 and viral load by applying bar graphs.

Specific Objective 2

The association between plasma fat and absolute CD4 count: Plasma fat (cholesterol, LDL-c, HDL-c, triglyceride) and BMI were independent variables while absolute CD4+ count was the dependent variable. Multiple linear regression was applied to determine the association between plasma fat and absolute CD4 count.

Specific Objective 3

The association between plasma fat and viral load: Plasma fat (cholesterol, LDL-c, HDL-c, triglyceride) and BMI were the independent variables while viral load was the dependent variable. Multiple linear regression was applied to determine the association between plasma fat and viral load.

Specific Objective 4

The association between plasma fat and opportunistic infection: Plasma fat and BMI were the independent variables while opportunistic infection was the dependent variable. In order to measure opportunistic infections we first developed a variable called OpIn. We then coded the variable OpIn as 1 to represent the presence and 0 as the absence of opportunistic infection. Based on the coding and the respective label we applied multiple linear regression to determine the associations between the dependent and independent variables.

Specific Objective 5

The association between BMI and CD4 count or viral load. BMI was the independent variable while CD4 and viral load were the dependent variables.

In all the regression models, we adjusted for age, sex, smoking and alcohol status.

STATA version 12 was used for statistical analysis.

4.93 Ethical Considerations

Human Subjects

This proposal and consent forms were granted approval by the Tropical Disease Research Centre Ethics Committee (TDRC) and the Ndola Teaching Hospital management in Ndola, Zambia. The study registration number is **STC/2018/2 and TDRC-IRB no. 00002911.** The study was further granted approval by the Zambia National Health Research Authority (ZNHRA) in Lusaka, Zambia.

Details of the study were carefully discussed with the study candidates identified from the Ndola Teaching Hospital ART program. The participants were asked to read and sign the consent form. If the participant and legal guardian were unable to read, the process for consenting illiterate participants, as defined by the TDRC Research Ethics Committee, was followed. Written informed consent was obtained from all participants.

Risks to the subjects: The level of risk associated with this research was expected to be minimal.

Minor bleeding and bruising may have been experienced from blood draws.

Subjects were recruited from persons who already knew their status and had been subjected to the pre-enrollment procedures.

4.94 Primary and Secondary study outcomes

From the 174 research subjects we anticipated to draw conclusive results suggesting the potential role of fat in improving clinical outcomes based on its association with CD4+ counts and with viral load. Further, study findings are expected to present grounds to justify the need to establish and strengthen the nutritional component in HIV care through regular monitoring and determination of lipid and BMI status and as being fundamental in the comprehensive management of the HIV/AIDS population. Findings from this study could therefore have a significant impact on people living with HIV/AIDS and initiating ART in Zambia and worldwide.

4.95 Summary

The study was a cross sectional design in which quantitative methods were applied to determine the relationship between the independent and outcome variables with the primary goal being to analyze and represent the interaction through statistical analyses. The main independent variables studied were total cholesterol, and its subtypes, triglyceride and BMI while the main outcome variables were CD4 count, viral load and opportunistic infection. The target population were adult HIV/AIDS patients receiving care from the HIV clinic, who were eligible and consented to participate in the study. A total of 174 participants who were selected through a simple random sampling technique were recruited and followed up for the study. The Zambian Ministry of Health certified and standardized questionnaires in HIV care were adopted and applied to derive information on history taking, physical assessments and anthropometric measurements. The FacsCount machine was used for CD4 count measurement, while the Ampliprep and Taqman machine was used for viral load measurement.

The Cobas C111 machine was utilized for plasma fat analyses. The data collected was managed through a secure and confidential hard copy filing and the electronic patient information tracking and processing systems. The baseline characteristics of participants by gender were analyzed using Wilcoxon rank sum test for continuous variables and chi-square test for categorical variables. The main research question was addressed by establishing the association between plasma fat and CD4 count and that between plasma fat and viral load adjusted for potential confounders using the multiple linear regression model. The statistical software package used to analyze the data was STATA version 12.

Ethical approval was sought and granted by the TDRC Research Ethics Committee in Ndola and the National Health Research Authority in Lusaka, Zambia.

Chapter Five: Research Findings

5.0 Introduction

This chapter reports outcomes following the analyses based on the data collected during the study. Results are presented in figures, tables, diagrams, and drawings, for ease of interpretation, understanding, reading and discussion. In this study, results will be presented in such a way that they correspond to the general and specific objectives (Dube O, 2011)

5.1 Descriptive Data:

Objective 1

Table 5.11. Baseline Characteristics

Variable	Female N= 107 (61%)	Male N= 67 (39%)	p
Age (years)	37 (34.5, 38.7)	40(37.5, 42.4)	0.02
BMI (kg/m2)	22.9 (20.4, 27.5)	21 (18.8, 23.9)	0.01
CD4+count (cells/ul)	357 (231, 543)	245.5 (167.5, 407.5)	0.002
Viral Load (copies/ml)	355 (20, 6770)	254 (23, 2694)	0.84
Total Cholesterol (mmol/l)	3.86 (3.02, 4.62)	3.53 (3.06, 4.61)	0.65
Triglyceride (mmol/l)	1.19 (0.87, 1.51)	0.96 (0.71, 1.60)	0.25
LDL-C (mmol/l)	2.31 (1.58, 2.90)	1.86 (1.36, 2.80)	0.19
HDL-C (mmol/l)	1.33(1.13, 1.51)	1.4 (1.21, 1.55)	0.15
Opportunistic infection			
Present	15 (15.2%)	17(27%)	0.06
Co-morbidity			
Present	14 (14.1%)	2 (3.23%)	0.02
Smoke Status			
Yes	2 (3.08%)	9 (23.1%)	0.001
Alcohol Status			
Yes	16 (16.2%)	23 (37.1%)	0.003

Values are median (interquartile range) unless otherwise stated, BMI= Body Mass Index, CD4+=Cluster Differential, LDL= Low Density Lipoprotein-cholesterol, HDL= High Density Lipoprotein-Cholesterol, TC= Total Cholesterol.

Figure 5.12. Plasma fat profiles by visit

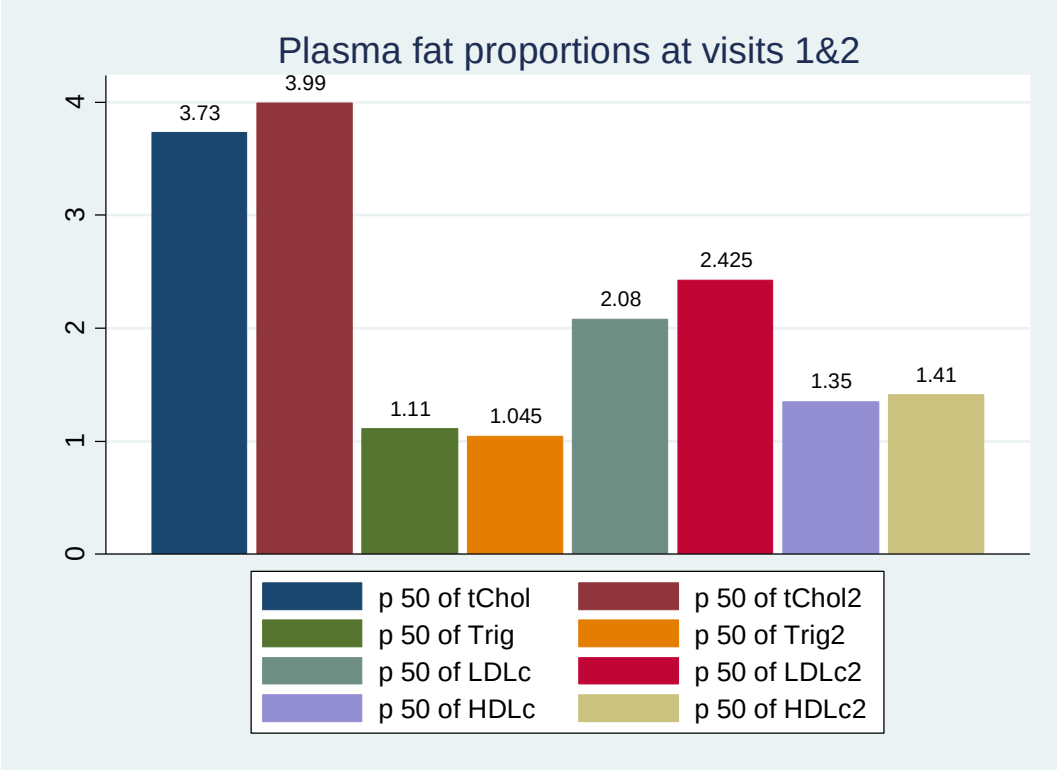


Figure 5.13. CD4+ count profiles by visit

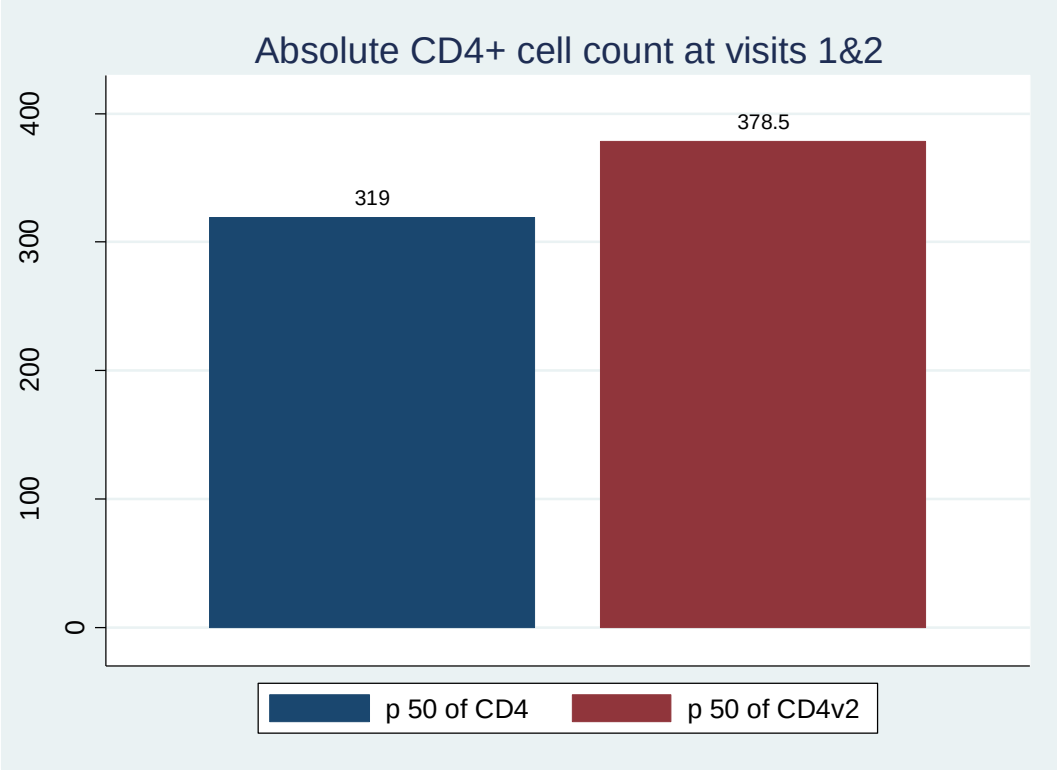
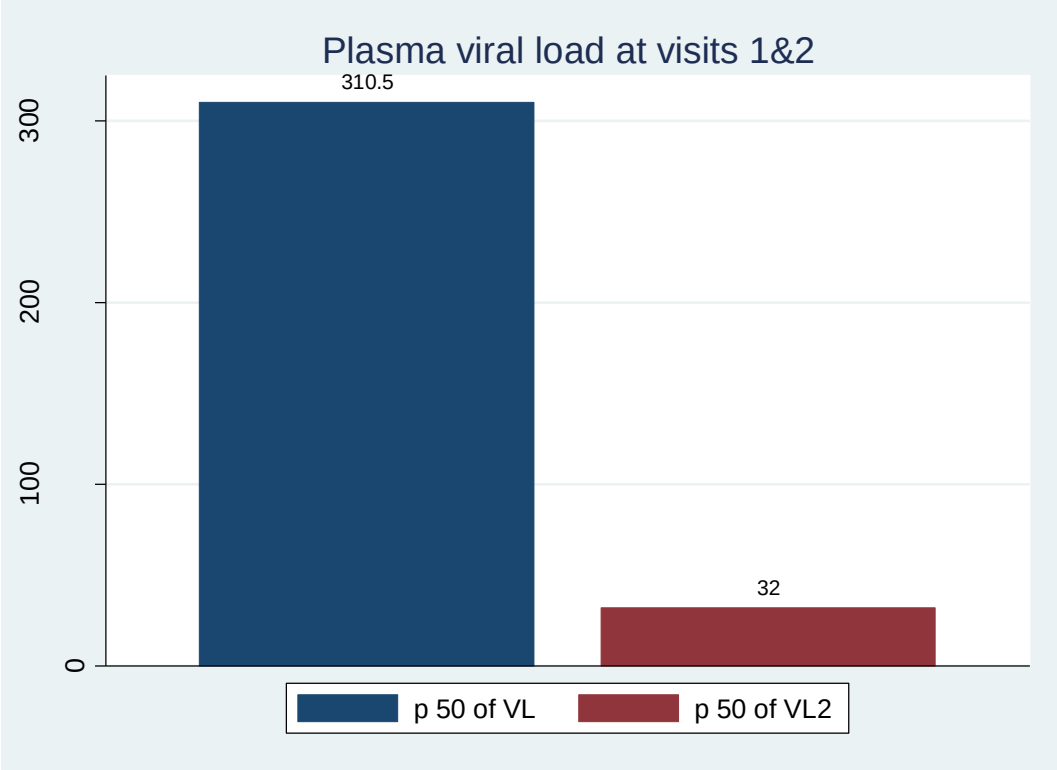


Figure 5.14. Plasma viral load profile by visit



5.2 Analytical Data

Objective 2

Table 5.21.

Multiple linear regression taking CD4+ as the dependent variable on a log scale

Independent Variables	Unadjusted estimates (95% CI)			p-value	Adjusted estimates (95%CI)			p-value
tChol2	0.04	(-0.08	0.16)	0.49	-0.79	(-2.21	0.64)	0.28
Trig2	-0.08	(-0.34	0.18)	0.54	0.06	(-0.35	0.47)	0.77
HDLc2	0.04	(-0.93	1.02)	0.93	0.69	(-0.95	2.34)	0.41
LDLc2	0.05	(-0.08	0.18)	0.41	0.81	(-0.62	2.24)	0.26
lnVL2	-0.10	(-0.14	-0.05)	0.00	-0.09	(-0.14	-0.03)	0.01
BMI	0.05	(0.03	0.08)	0.00	0.04	(0.00	0.07)	0.03
Age_n	0.00	(-0.02	0.01)	0.96	-0.01	(-0.03	0.01)	0.32
Gender	0.40	(0.09	0.71)	0.01	0.34	(-0.06	0.74)	0.10
SmoSt	-0.04	(-0.52	0.43)	0.86	0.22	(-0.41	0.85)	0.49
AlSt	-0.21	(-0.57	0.14)	0.23	-0.19	(-0.65	0.27)	0.42

Using multiple linear regression, adjusted for Age, BMI, Gender, Smoke Status and Alcohol Status by visit 2. CD4+=Cluster differentiation 4, tChol= total Cholesterol (mmol/l), Trig=Triglyceride (mmol/l), LDL= Low Density Lipoprotein-Cholesterol (mmol/l), HDL= High Density Lipoprotein-Cholesterol (mmol/l), VL= Viral Load (copies/ml), BMI=Body Mass Index (kg/m^2), SmoSt=Smoke Status, AlSt=Alcohol Status

Objective 2

Table 5.22.

Multiple linear regression taking CD4+ as the dependent variable on a log scale by female gender

Independent variables	Adjusted estimates (95% CI)			p-value
tChol2	-6.43	(-19.50	6.65)	0.33
Trig2	1.13	(-1.54	3.79)	0.40
HDLc2	6.50	(-6.58	19.58)	0.32
LDLc2	6.43	(-6.63	19.48)	0.33
lnVL2	-0.11	(-0.18	-0.04)	0.00
BMI	0.04	(0.00	0.08)	0.04
Age_n	0.00	(-0.03	0.02)	0.68
SmoSt	-0.02	(-1.38	1.35)	0.98
AlSt	-0.19	(-0.90	0.51)	0.59

Using multiple linear regression, adjusted for Age, BMI, Smoke Status and Alcohol Status by visit 2. CD4+=Cluster differentiation 4, tChol= total Cholesterol (mmol/l), Trig=Triglyceride (mmol/l), LDL= Low Density Lipoprotein-Cholesterol (mmol/l), HDL= High Density Lipoprotein-Cholesterol (mmol/l), VL= Viral Load (copies/ml), BMI=Body Mass Index (kg/m^2), SmoSt=Smoke Status, AlSt=Alcohol Status

Objective 3

Table 5.23.

Multiple linear regression taking viral load as the dependent variable on a log scale

Independent variables	Unadjusted estimates (95% CI)			p-value	Adjusted estimates (95%CI)			p-value
tChol2	-0.04	(-0.48	0.40)	0.86	-3.29	(-8.40	1.83)	0.21
Trig2	0.16	(-0.80	1.13)	0.74	0.51	(-0.95	1.97)	0.49
HDLc2	2.72	(-0.80	6.24)	0.13	7.31	(1.61	13.02)	0.01
LDLc2	0.05	(-0.08	0.18)	0.41	3.22	(-1.91	8.35)	0.22
lnCD4v2	-1.39	(-2.01	-0.78)	0.00	-1.11	(-1.87	-0.35)	0.01
BMI	-0.09	(-0.19	0.01)	0.09	-0.07	(-0.19	0.06)	0.29
Age_n	-0.05	(-0.10	0.00)	0.05	-0.05	(-0.11	0.01)	0.10
Gender	0.46	(-0.66	1.59)	0.42	1.05	(-0.40	2.49)	0.15
SmoSt	0.03	(-1.70	1.77)	0.97	-0.76	(-3.03	1.51)	0.51
AlSt	1.28	(0.01	2.55)	0.05	1.99	(0.39	3.60)	0.02

Using multiple linear regression, adjusted for Age, BMI, Gender, Smoke Status and Alcohol Status by visit 2. CD4+=Cluster differentiation 4, tChol= total Cholesterol (mmol/l), Trig=Triglyceride (mmol/l), LDL= Low Density Lipoprotein-Cholesterol (mmol/l), HDL= High Density Lipoprotein-Cholesterol (mmol/l), VL= Viral Load (copies/ml), BMI=Body Mass Index (kg/m^2), SmoSt=Smoke Status, AlSt=Alcohol Status

Objective 3

Table 5.24.

Multiple linear regression taking VL as the dependent variable on a log scale by female gender

Independent variables	Adjusted estimates (95% CI)			p-value
tChol2	4.96	(-42.83	52.75)	0.84
Trig2	-1.59	(-11.30	8.12)	0.74
HDLc2	-0.35	(-48.19	47.48)	0.99
LDLc2	-4.93	(-52.64	42.78)	0.84
lnCD4v2	-1.47	(-2.42	-0.52)	0.00
BMI	-0.03	(-0.18	0.11)	0.66
Age_n	-0.07	(-0.15	0.01)	0.09
SmoSt	-1.44	(-6.36	3.49)	0.56
AlSt	2.19	(-0.29	4.68)	0.08

Using multiple linear regression, adjusted for Age, BMI, Smoke Status and Alcohol Status by visit 2. CD4+=Cluster differentiation 4, tChol= total Cholesterol (mmol/l), Trig=Triglyceride (mmol/l), LDL= Low Density Lipoprotein-Cholesterol (mmol/l), HDL= High Density Lipoprotein-Cholesterol (mmol/l), VL= Viral Load (copies/ml), BMI=Body Mass Index (kg/m^2), SmoSt=Smoke Status, AlSt=Alcohol Status

Objective 4

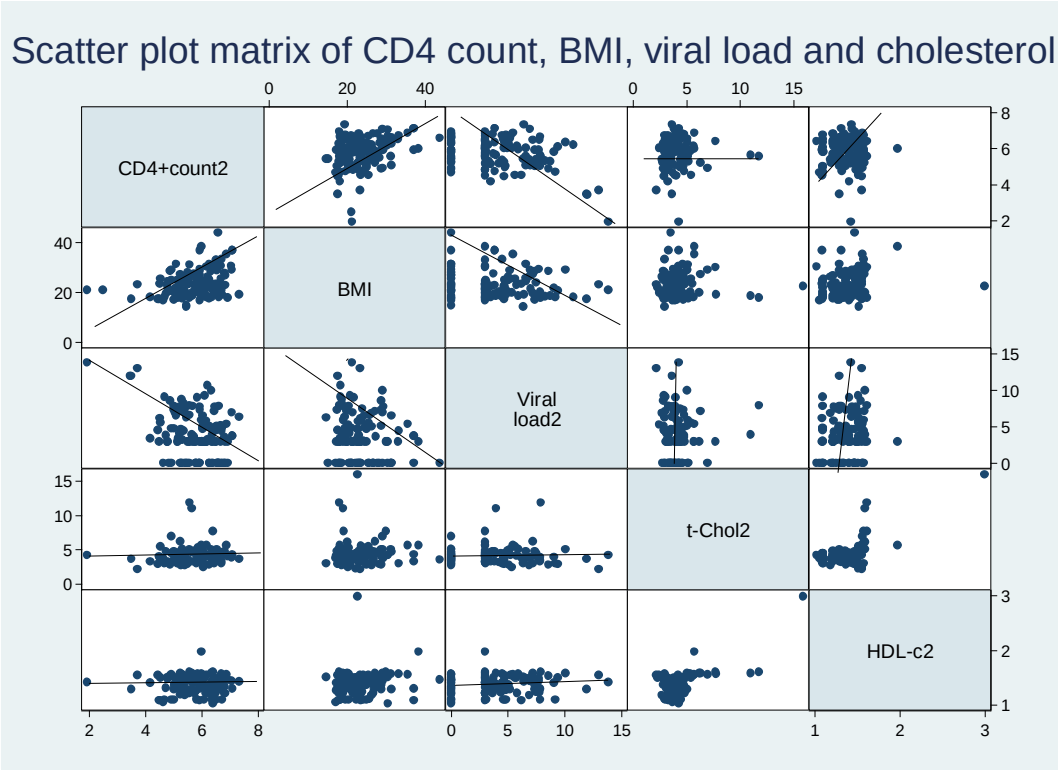
Table 5.25.

Multiple linear regression taking OpIn as the dependent variable

Independent variables	estimates	Unadjusted (95% CI)		p-value	estimate	Adjusted (95%CI)		p-value
tChol2	0.00	(-0.05	0.04)	0.88	0.24	(-0.50	0.99)	0.52
Trig2	0.04	(-0.04	0.12)	0.32	-0.10	(-0.31	0.11)	0.34
HDLc2	0.10	(-0.24	0.44)	0.58	-0.35	(-1.20	0.51)	0.42
LDLc2	-0.01	(-0.06	0.04)	0.67	-0.27	(-1.01	0.48)	0.48
lnVL2	0.01	(-0.01	0.04)	0.19	0.01	(-0.02	0.04)	0.64
lnCD4v2	-0.17	(-0.25	-0.09)	0.00	-0.09	(-0.21	0.02)	0.11
BMI	-0.01	(-0.03	0.00)	0.04	-0.01	(-0.03	0.01)	0.45
Age_n	0.00	(-0.01	0.00)	0.48	0.00	(-0.01	0.01)	0.72
gender	-0.12	(-0.25	0.00)	0.06	-0.12	(-0.33	0.09)	0.27
SmoSt	-0.10	(-0.30	0.10)	0.32	-0.24	(-0.57	0.09)	0.15
AlSt	0.04	(-0.11	0.19)	0.58	0.12	(-0.12	0.36)	0.32

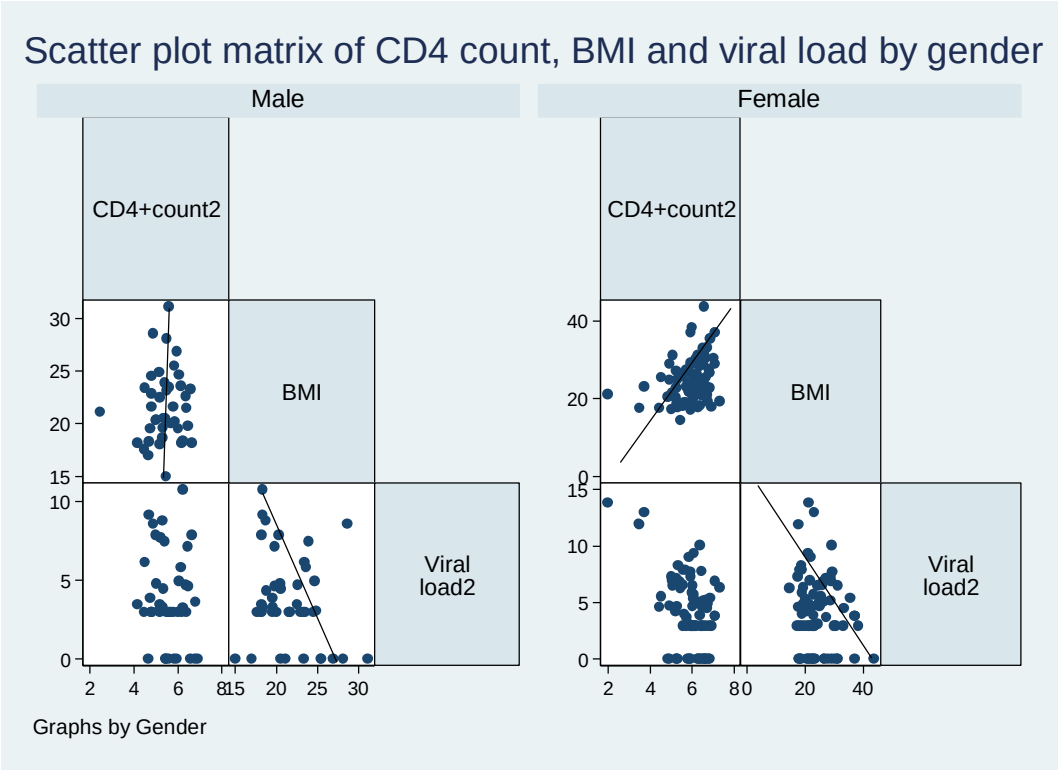
Using multiple linear regression, adjusted for Age, Gender, BMI, Smoke Status and Alcohol Status by visit 2. CD4+=Cluster differentiation 4, tChol= total Cholesterol (mmol/l), Trig=Triglyceride (mmol/l), LDL= Low density lipoprotein-cholesterol (mmol/l), HDL= High density lipoprotein-cholesterol (mmol/l), VL= Viral load (copies/ml), BMI=Body mass index (kg/m^2), SmoSt=Smoke Status, AlSt=Alcohol Status, OpIn=Opportunistic Infection

Figure 5.26. Scatter plot matrix



Objective 5

Figure 5.27. Hemi-Scatter plot matrix



5.3 Results Interpretation

Objective 1.

5.31 Baseline Characteristics

The description of the study population is as suggested in table 5.11. The baseline characteristics of the population under study including age, physical measurements, lipid profiles, the immunological and virological status are values expressed as median (interquartile range) unless stated otherwise. The clinical status parameters being the presence or absence of opportunistic infections and/or comorbidities and the social status parameters of smoking and alcohol are measures reported in percent proportions.

The total number of subjects whose results were available for analysis in the study was 174. Of the 174 subjects, 107 (61%) were female and 67(39%) were males. The study population were generally young, with males being older [mean=40; interquatile (37.5, 42.4) years] than females [mean=37; interquatile (34.5, 38.7) years], p=0.02. The BMI was within the normal range for both male and female gender, although notably higher in the females [median=22.9; interquatile (20.4, 27.5) kg/m2] than in the males [median=21; interquatile (18.8, 23.9) kg/m2], p=0.01. The median CD4+ counts were found to be lower than the lower limit of the normal for the laboratory reference range of (500-1500 cells/ul) in both gender. The value was especially lower in the males [median=245.5; interquatile (167.5, 407.5) cells/ul] than in the females [median=357; interquatile (231, 543) cells/ul], p=0.002. The viral loads were found to be relatively higher than the expected for optimal suppression in both gender. The females were especially more poorly suppressed [median=355; interquatile (20, 6770) copies/ml] than the males [median=254; interquatile (23, 2694) copies/ml], though falling short of statistical significance, p=0.84.

The lipid profiles were generally within normal for the laboratory reference ranges for both the male and female gender respectively. However, the total cholesterol [median=3.86; interquatile (3.02, 4.62) mmol/l], triglyceride [median=1.19; interquatile (0.87, 1.51) mmol/l] and LDL-c [median=2.31; interquatile (1.58, 2.90) mmol/l] concentrations were relatively higher in the females than the males [median=3.53; interquatile (3.06, 4.61) mmol/l], [median=0.96; interquatile (0.71, 1.60) mmol/l] and

[median=1.86; interquatile (1.36, 2.80) mmol/l] respectively. Conversely, the HDL-c concentration were found to be relatively higher in the male [median=1.4; interquatile (1.21, 1.55) mmol/l] than the female gender [median=1.33; interquatile (1.13, 1.51) mmol/l]. However, the gender disparities in the lipid panel did not suggest statistical significance.

The results per clinical status revealed relatively more males [17 (27%)] than females [15 (15.2%)], p= 0.06 presenting with opportunistic infections and more females [14(14.1%)] than males [2 (3.23%)], p=0.02 presenting with co-morbidities. Results per social status suggested relatively more male [23 (37.1%)] than females [16(16.2%)], p=0.003, taking alcohol and more males [9 (23.1%)] than female [2(3.08%)], p=0.001 smokers.

Objective 1

5.32 Plasma fat, CD4 and viral load profiles by visit

Figure 5.12 is a bar graph depicting the plasma fat proportions at visit 1 and visit 2. The result suggests an incremental trend in the plasma fat proportions from visit 1 to visit 2 for all the plasma fat types except for the triglycerides. Similarly, an upward trend is suggested for the median CD4+ cell count at visit 1 (319) compared with that for visit 2 (378.5) in figure 5.13, while figure 5.14 depicts a decremental trend in the median viral load reported for visit 1 (310.5) compared with that at visit 2 (32).

Objective 2

5.33 Multiple linear regression with CD4+ count as dependent variable

Results in table 5.21 suggested no statistically significant association between the plasma fat types and absolute CD4+ count. However a statistically significant inverse association was found between absolute CD4+ count and viral load in both the unadjusted [Coef=-0.10; 95% CI (-0.14, -0.05), p=0.00] and adjusted [Coef=-0.09; 95% CI (-0.14, 0.03), p=0.01] models respectively and also a significant positive association between CD4+ count and the body mass index in both the unadjusted [Coef=0.05; 95% CI (0.03, -0.08), p=0.00] and adjusted [Coef=0.04; 95% CI (0.00, 0.07), p=0.03] models respectively.

In table 5.22, similar results suggesting a significant inverse association between absolute CD4+ count and viral load [Coef=-0.11; 95% CI (-0.18, -0.04), p=0.00] and a significant positive association between CD4+ count and the body mass index [Coef=0.04; 95% CI (0.00, 0.08), p=0.04] were reported in the adjusted model for the female gender.

Objective 3

5.34 Multiple linear regression taking viral load as the dependent variable

In table 5.23, results show statistically significant inverse association between plasma viral load and CD4+ count in both the unadjusted [Coef=-1.39; 95% CI (-2.01, -0.78), p=0.00] and adjusted [Coef=-1.11; 95% CI (-1.87,-0.35), p=0.01] models respectively and also a significant positive association between viral load and alcohol status in both the unadjusted [Coef=1.28; 95% CI (0.01, 2.55), p=0.05] and adjusted [Coef=1.99;95% CI (0.39, 3.60), p=0.02] models respectively. Although a significant positive association was reported between viral load and HDL-c in the adjusted estimates [Coef=7.31:95% CI (1.61, 13.02), p=0.01] the association was insignificant in the unadjusted model [Coef=2.72; 95% CI (-0.80, 6.24), p=0.13]

Objective 4

5.35 Multiple linear regression taking opportunistic infection as the dependent variable

Results in table 5.25 suggested statistically significant inverse associations between opportunistic infections and CD4+ count [Coef=-0.17; 95% CI (-0.25, -0.09), p=0.00] and that between opportunistic infections and BMI [Coef=-0.01; 95% CI (-0.03, 0.00) p=0.04] in unadjusted estimates. The association is however, weaker when adjusted for. Results in both the main and the by gender regression analyses did not reveal statistically significant associations between plasma fat types and opportunistic infections. However, results in the by gender regression analyses suggested a consistent inverse interaction involving most of the plasma fat types and BMI with opportunistic infections.

Objective 5

5.36 Scatter plot matrix of CD4+ count, viral load, cholesterol and BMI

Figure 5.26 is a scatter plot matrix depicting a positive correlation between HDL-c and CD4 + count, an ill-defined correlation between HDL-c and viral load, near neutral correlations between t-cholesterol and CD4+ count and that between t-cholesterol and viral load. The graph further suggests the positive and inverse correlations between BMI and CD4+ count and that between BMI and viral load respectively. Figure 5.27 is a hemi-scatter plot matrix signifying a positive correlation between BMI and CD4+ count and an inverse correlation between BMI and viral load by gender.

Chapter Six: Discussion

6.0 Introduction

In this chapter, the study focuses on the relevance of the outcomes and how the study findings correspond with other studies in the given field. The chapter thus speaks to the literature review and the introductory aspects of the study. According to Dube O, (2011), data interpretation uses literature review to describe the past and present status of the stated problem and requires deep knowledge of the problem area. In this study we will seek to provide rational explanations for the study findings by relating the results to findings from previous studies and provide citations as appropriate per literature review. We will also endeavor to report some study limitations in this section.

6.1 Discussion

6.11 Demographics

The finding of a relatively young study population of the median age (37years) for females and (40 years) for the males, does confirm prior reports of worrying proportions of young persons presenting with HIV infection in our setting. The study statistics show that females [107(61%)] are particularly more affected than males [67(39%)]. However, the trend may also be explained by the relatively higher proportion of females [50.5%] than males [49.5%] in the general population (Kemp S, 2021). It is also possible that the disparity may be due to the poor health seeking behavior in males compared to females. The prevalence of HIV among adults aged 15-59 years in Zambia is 12% of which 14.6% are females and 9.6% males (MOH, 2019) and our study finding of a relatively young population is within the reported age range. Also, adolescents and young adults of ages 15-24 years are reportedly more likely to engage in risky sexual behaviors than older adults and have less frequent contact with the health care system (Hervish A, 2012).

This is obviously a challenge to health care but is a wakeup call presenting an opportunity for strategic approaches tailored towards addressing health matters and lifestyle issues affecting the adolescents and young adults.

6.12 Plasma Fat Profiles

The trend suggesting generally improved plasma fat profiles from visit 1 to visit 2 is in keeping with findings from prior studies suggesting increased nutrient uptake (including fats) associated with enhanced appetite and resolving HIV enteropathy following the initiation of antiretroviral therapy (Kotler DP, et al, 2005, Weiser SD, et al, 2010). The tight epithelial junctions, as well as the local immune system of the GI tract, protect against pathogenic organisms. However, in the face of HIV infection normal defences are disrupted leading to a wide range of clinical and pathogenic consequences (Crum-Cianflone N F, 2010). The administration of HAART not only improves the systemic immune system, but also the local cellular immunity of the GI tract and therefore being the cornerstone for both the prevention and treatment of opportunistic GI infections and HIV enteropathy (Cello JP and Day LW 2009). Nutrient loses which may occur due to malabsorption, diarrhea and vomiting are therefore expected to reduce through the suggested mechanisms and hence the reported trends. It is also argued that while cART in HIV-1 infected subjects generally allows for immune reconstitution in peripheral blood, reconstitution of the GI tract occurs at a much slower pace and both immunological and structural abnormalities persist in the GI tract (Mudd JC and Brenchley JM, 2016). Further, because chronic gut inflammation is also characteristic of HIV-1 and SIV infection, the question of whether significant shifts in the gut microflora composition (dysbiosis) occurs in HIV-1 and SIV infection and how this can influence disease pathology has also been a subject under examination by researchers (Vujkovic-cvijin I, et al, 2013, Dillon SM, et al, 2014).

6.13 Body mass index (BMI) profiles

The median BMIs of 22.9kg/m2 and 21kg/m2 for females and males respectively and the 17% proportion of subjects with low BMI in our study suggests relatively improved

BMI profiles in contrast with prior findings in other settings (Uthman OA, 2008, Weiser SD et al 2009), where malnutrition in the form of low BMIs ranging from 10% to 33% in sub-Saharan Africa has been reported. Similar findings have been revealed in a survey conducted in Lusaka, Zambia where individuals with a low BMI represented a significant proportion of those presenting for HIV care in the region. In this survey, 33% of patients starting cART had a BMI < 18.5kg/m2 and 9% had a BMI < 16.0 kg/m2 (Koethe JR, et al, 2010). Further contrast can be drawn with a study conducted by Nyirenda et al, (2015), in which the median BMI of 19.6 kg/m2 for both gender was reported. It is also possible however, that the differences in the socio-economic status of the subjects in the settings under comparison may in part explain the noticeable disparities in the BMIs.

6.14 CD4+ count and Viral Load profiles

The observed incremental trend for the CD4+ count which corresponded with the trend for plasma fat and a downward trend in the viral load may suggest a plausible positive and inverse interaction with plasma fat respectively. The trend may support prior study findings that cells of the immune system and in individuals with hypercholesterolemia had greater phagocytic activity, more circulating lymphocytes, more total T cells, more CD8+ T cells, more immunoglobulin production, more proliferation and differentiation and migration of lymphocytes than from individuals with lower cholesterol levels (Hannedouche S, et al, 2011, Liu C, et al, 2011, Adal M, et al, 2018). Studies involving fatty acids reveal similar findings. In macaque monkeys, arachidonic acid is associated with an increase in the T-helper 17 (TH 17) CD4+ cell compartment (Kabagambe EK, et al, 2016, Filteau S, et al, 2015) Which is known to lower the viral loads for the simian immune virus (Hartigan-O'Connor DJ, et al, 2012).

Consistent findings have also been reported in a study among ART-naïve HIV patients in Zambia where higher plasma arachidonic acid levels were found to be associated with better CD4+ cell counts and other markers of improved survival (Nyirenda CK, et al, 2015)

6.15 Regression for CD4+ count versus plasma fat and BMI

The finding of the no statistically significant association between absolute CD4+ count and plasma fat types in our study is in contrast with findings in some prior studies in which significant positive and inverse associations between some plasma fat types and CD4+ count and that between plasma fat type and viral load have been reported respectively. For instance, in a study by Adal M, et al, 2018, gender and serum total cholesterol were found to be associated and independent predictors of HIV RNA load and CD4+ cell count and/or WHO clinical stage. The study reported significant lower HIV RNA load and better CD4+ T cell count in women and study participants with higher total cholesterol. This is consistent with the understanding that the inhibition of HIV replication in study participants with high serum total cholesterol could be due to the production of oxysterols (Liu S, et al, 2013, Cyster JG, et al, 2014, Adal M, et al, 2018)

Oxysterols are oxidized forms of cholesterol involved in various functions such as the control of gene expression, signal transduction and cell migration (Choi C and Finlay DK, 2020). They can be obtained through the diet, particularly in cholesterol-rich foods or synthesized in various tissues and cells by distinct cholesterol hydroxylases and/or by auto-oxidation with reactive oxygen species (Russell, D.W, 2000, Brown, A.J. and Jessup,W, 2009).

The observation that BMI was significantly and positively associated with absolute CD4+ count in both the unadjusted and adjusted estimates (tables 5.21 & 5.23) is in keeping with findings from prior studies suggesting a positive role in immune recovery. In a study from South Africa by Barth RE et al, 2008, a BMI in the lowest quartile(< 17.1kg/m2) was associated with failure to achieve a CD4+ count ≥ 200 cells/ul at 12 months (Barth RE, et al 2008). A study from Singapore found no association between BMI and the magnitude of CD4+ cell recovery, but a low BMI was a significant independent predictor of death for several years following ART initiation (Paton NI, et al, 2006).

A study by Koethe JR et al, (2010), revealed statistically significant inverse association between baseline BMI and the post-6 month hazard for mortality only among those patients with CD4+ count < 100 cells/ ul increase in the first 6 months of ART. Higher baseline BMI have also been shown to predict better immune reconstitution in HIV infected patients after HAART initiation according to a study by Li X, et al, (2019). In this study, patients with a higher baseline BMI had an independently positive effect on 30-month CD4+ T lymphocyte recovery (p=0.028), but not 12 month CD4+ T lymphocyte gain (p=0.104).

6.16 Regression for Viral Load versus Plasma Fat and BMI

With the exception of the HDL-c, plasma fat types and BMI were not significantly predictive of plasma viral load status. Although HDL-c was positively associated with plasma viral load in the adjusted estimates, the result was not statistically significant in the unadjusted for estimates. Most prior studies suggest an inverse interaction between HDL-c and viral load. Further, the positive though not statistically significant association between HDL-c and absolute CD4+ count reported in our study is in concordance with some findings from prior studies. For instance, a study by Levy ME, et al, 2018 has shown that immunosuppression and viremia were each independently associated with higher atherogenic cholesterol concentrations among older HIV-infected patients than among younger patients. The study also found that the association between uncontrolled viremia and having lower HDL-c concentrations was stronger when patients had low CD4+ cell counts. Similar findings have been reported in another study where a high prevalence of dyslipidemia characterized by low-HDL-c and associated with a high viral load and low CD4+ cell count was found (Ngoroge A, et al, 2017).

An invitro study has also shown that beta-estradiol inhibited HIV-1 replication in human peripheral blood lymphocytes by inhibiting target cell infection that involves cell entry through higher expression of chemokines (Rodriguez-Garcia M, et al, 2013). Despite lack of statistical significance, the BMI was found to be inversely associated with viral load, a finding plausibly suggesting its potential role in viral suppression.

In the alcohol status versus viral load regression, our study revealed significant positive association between alcohol use and viral load. Prior studies elsewhere suggest mixed outcomes. A Kenyan study for example has shown that while targeted interventions to address alcohol use could impact ART adherence, the effect may not be large enough to improve viral suppression for the target population where few women reported harmful or dependent alcohol use (Long JE, et al, 2020). In a Florida study, heavy drinking was significantly associated with poor viral suppression and ART non adherence, while binge drinking was associated with suboptimal ART adherence (Cook RL, et al 2017).

6.17 Regression for Opportunistic Infections versus Plasma Fat and BMI

There was no statistically significant association between plasma fat types and opportunistic infections. However, in both the overall and the gender based regression analyses, the study revealed a consistent inverse interaction involving most of the plasma fat types and BMI with opportunistic infections. This finding may suggest a potential role of plasma fat or BMI in immune mechanisms or viral suppression and corresponding clinical outcomes, a subject for further exploration.

6.2 Study limitations

As a result of attrition following the lost to follow-up of some subjects by visit 2, the power of the study could potentially be limited to detect a statistically significant association especially for those interactions where significance may not have been suggested. The study was potentially prone to reporting bias which may arise from failure by respondents to report the truth. This could have been intentional for example due to social desirability where subjects would prefer to report good much more than bad attributes toward social status such as smoking or alcohol use. It may also have been unintentional such as when the respondent fails to recall or report clearly, responses toward the clinical assessments. The study may also be prone to measurement error which may arise during the testing process of the samples for the variables of interest.

Chapter Seven: Conclusion and Recommendations

7.0 Introduction

This chapter presents a summary of the most relevant answers to the research questions or objectives based on the study findings. The findings are used to recommend what can be done, to improve social actions, policies, activities and behavior change. However, some of the outcomes which may constitute new findings and knew knowledge may not fit into this format and are therefore recommended as areas needing further research (Dube O, 2011).

7.1 Conclusion

1. The typical plasma fat profiles in the study cohort of adult HIV/AIDS patients presenting for care were generally within normal and suggested a positive and inverse association with CD4 count and viral load respectively
2. Total plasma cholesterol, LDL-c, HDL-c and triglycerides were not significantly predictive of CD4 count. However, the consistent inverse interaction between total cholesterol and CD4 count in the overall and the by gender models may favor a potential predictive role
3. Total plasma cholesterol and its subtypes were not significantly predictive of viral load and although HDL-c was shown to be positively associated with viral load in the adjusted estimates, the association was insignificant in the unadjusted model
4. Plasma fat status was not significantly predictive of the prevalence of opportunistic infections. However, there was an overall and by gender consistent inverse interaction suggested between plasma fat and opportunistic infections
5. Body mass index was found to be a significant positive predictor of CD4 count (overall), and more substantively so by female gender and also an independent predictor of opportunistic infections. The association with viral load was inverse though not statistically significant

In summary, while plasma fat may be potentially predictive of immune recovery, viral suppression and clinical status, a healthy BMI, rather than the role of individual or a selected group of plasma fats appears to be more beneficial towards positive clinical outcomes

Novelty and Knowledge gap resolutions: The finding that BMI is a significant predictor of CD4+ counts and opportunistic infections and a potential predictor of viral load is novel. Study findings also suggest evidence of the potential role of some plasma fat in immune augmentation and viral suppression based on their consistent positive and inverse interaction with CD4+ count and viral load respectively, despite falling short of statistical significance. Based on the established evidence, especially with regard to the role of a healthy BMI, a clinical trial involving appropriate nutritional supplementations would provide more grounds for justification.

7.2 Recommendations

1. Inform hospital protocols, to underscore the need for regular monitoring of lipid profiles and consistent BMI measurements as key components in the management of HIV/AIDS patients. [Clinicians' role]
2. Ensure availability of quality assured biochemistry labs and anthropometric tools to promote regular monitoring for plasma and body fat respectively in order to achieve healthy BMIs and lipid profiles. BMI estimates should be supplemented by other measures of adiposity and weight to enhance accuracy. [Hospital Management's role]
3. Establish and strengthen dietetic units in HIV/AIDS care facilities to provide appropriate nutritional support services including food supplements, health education and promotion tailored towards appropriate dietary prescriptions and lifestyle adjustments as part of the comprehensive care package for our HIV/AIDS patients. [Zambian Ministry of Health and Hospital Management's role]
4. Lobby for funding support tailored towards the nutritional needs of the HIV/AIDS patients, to be specified within the ceiling of nutrition in the hospital's budgetary allocation. [Hospital Management's role]
5. Alternatively engage the private sector in a public, private partnership arrangement to supplement government support towards the nutrition programs in HIV/AIDS care. [Zambian Ministry of Health and the Private Sector's role]

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APPENDICES

Appendix I

Consent Form

PID# _____

Research Title: A study relating Plasma Fat to Immunological, Virological and Clinical Status in Zambian Adults with HIV/AIDS

Principal Investigator: Christopher Nyirenda (MD)

Research Assistants: Dr Ray Handema, Dr Kennedy Gondwe, Dr Sandra Terry, Dr Justin Chileshe, Mr. Sydney Mwanza, Mr. Allen Chipipa et al.,

Sponsors: The Ministry of Health, Ministry of Higher Education and the Tropical Diseases Research Centre in Zambia

Introduction

You are being asked to participate in a research study to establish how plasma fats relate to your immune status (level of CD4 count) and viral load from the time you initiate anti-retroviral therapy. Fats are among important macronutrients for health which if consumed normally and in a balanced diet may have a role in the management of several diseases and may also be beneficial in the management of HIV/AIDS.

This is a consent form. It gives you information about this study. We want you to know the procedures, purpose and what is expected of you if you decide to join. If you decide to take part in this study after all the explanation about it, we will ask you to sign this consent form or make a thumbprint in front of a witness.

Page 1 of 7 Patients Initials/Thumbprint _____

Please note that participation in this study is entirely voluntary. Should you be willing to participate it is expected that you would remain in the study area for at least 3 months from the time of enrollment and to allow for a possible follow up to your home in the event of missed clinic visits.

.

PID# _____

Study Objectives

The main purpose of the study is especially:

1. To determine if plasma fat status would predict immune function and suggest a potential adjunct role of total fat and its sub-types for immune enhancement in HIV/AIDS patients
2. To determine if plasma fat status would predict HIV infection viral load to suggest an inverse interaction in confirming findings that some plasma fat types have the potential to inactivate HIV in laboratory models

This will further help us understand the role fats may play in the management of HIV/AIDS in addition to the role of anti-retroviral therapy.

Study procedures

If you agree to participate in this research study you will be required to sign for enrollment as a participant. Following your initial visit, you will be required to report back for review at 2-weeks and 12 weeks for study procedures but also for your ART reviews per routine clinical follow-up schedule.

At your first visit, you will be asked some medical questions and be subjected to physical examination to determine your general wellbeing and your nutritional status. You will also be assessed for suitability to start Septrin, Isoniazid or other drugs given to prevent opportunistic diseases prior to initiation of ART. Apart from screening for opportunistic infections you will also be required to do baseline organ function tests such as the liver and kidney function prior initiation of ART but also in the course of ART for monitoring purposes and per indication.

You will also be required to submit about a teaspoon of blood per parameter for testing to determine plasma fat status, CD4 count and viral load at baseline.

Risks and discomforts

When the study blood samples are being drawn, there is a small risk of pain, bruising or infection at the blood drawing site.

Benefits

As a subject you are expected to benefit from findings suggesting the potential role fats may play in the comprehensive management of HIV/AIDS. Findings from this study could also have a significant impact on People living with HIV/AIDS and initiating ART in Zambia and worldwide

Alternatives

There are no alternative treatments available other than receiving your HIV/AIDS treatment without the additional monitoring offered by this study.

Confidentiality

The information gathered during the study will be kept confidential and as required by the law. However, your doctor, clinic staff and study personnel will be able to inspect your medical records and have access to confidential information that identifies you by name. Before information about you is analyzed for research purposes, your name will be removed. It will be substituted with a code number so that your name can not be identified, and will be stored and secured on a computer.

Your name will not be used if any of the information about you is published in international journals or shared with the Zambian Government should it use the information from the study to improve the treatment of persons with HIV/AIDS.

Withdrawal from the study

You will be free to withdraw your consent and to discontinue participation in this project at any time without prejudice against further care that you may receive at this institution. Your participation in the study may be stopped at any time by the study doctor or the sponsor without your consent, for example, if the sponsor or investigator decides to discontinue the research.

Significant findings

You will be informed of any significant new findings that may develop in the course of the study which may also affect your willingness to continue in the research. The clinic staff will also ensure that an appropriate course of management is provided should need arise.

Cost of participation

There will be no cost to you for participating in the research. All study related examinations and laboratory tests will be provided at no cost during the 12 week study period.

You will be paid twenty Zambian kwacha (k20) for your transportation costs related to the additional clinic visit made for the research study. Should you withdraw your consent to participate before the additional visit, you will receive no payment.

Support toward research related injuries

It is unlikely that you will be injured in the course of your participation in the study. However, should there possibly be any injury, the clinic will provide immediate care as is locally available and may refer you to the relevant department at Ndola Teaching Hospital for further management. The care will be per standard available in the Government Health Institutions in Zambia.

PID# _____

Contact persons for questions

If you have any questions about the research or a research related injury, contact Christopher Nyirenda on 0977808749. He can also be reached at the Ndola Teaching Hospital, Department of Medicine, Postal Agency, Ndola. He can also be contacted through the clinical officer or nurse of the clinic. Should you have questions about your rights as a research participant, you may contact, the Secretary, TDRC Research Ethics Committee in Ndola.

PID# _____

Legal rights

You are not waiving any legal rights by signing the consent form

Your signature below indicates that you agree to participate in this study. You will receive a copy of this signed informed consent if you like.

Name of Participant	Signature or Thumbprint	Date
Witness (Print Name)	Witness (Sign)	Date
Person Obtaining Consent (Print Name)	Person Obtaining Consent (Sign)	Date

Appendix II:

Clinical Assessment Forms

CLINICAL ASSESSMENT FORM

Date D D / M M / Y Y

Province | District | Facility | Care card number | Serial no. | Check digit

Patient ID: P P - D D D - F F F - N N N N N N N N - S S S S S S - K

RECENT HISTORY ☐ No complaint *If there are any complaints note symptoms, duration, recurrence & other characteristics below.*

Always screen for TB:

	yes	no
Cough	☐	☐
Fever	☐	☐
Night sweats	☐	☐
Weight loss	☐	☐

☐ If 1 or more symptoms present, order Sputum for AAFB/GeneXpert or other

Gene part AAFB/ | ALT/AST | Creatinine | GCI | RBS/RST

CD4 Count | CD4 | Hb/HCT | Pregnancy | Other

PHYSICAL EXAM

Height (cm) | Weight (kg) | BMI (kg/m²)

BP / | Temperature o C | Heart rate/min | Respiratory rate/min

Vitals not taken because:
- ☐ Patient cannot stand
- ☐ No scale
- ☐ No staff available
- ☐ No Blood Pressure machine
- ☐ No thermometer

General exam (yes / no)
- Pallor ☐ ☐
- Jaundice ☐ ☐
- Edema ☐ ☐

PLAN Additional Notes

TB DRUG PRESCRIPTION

RHZE_____tabs od x____mo
S_____mg im od x____mo
EH_____tabs od x____mo
RHE_____tabs od x____mo
RFB_____mg od x____mo (if R-Rifabutin)
Vit B6____tabs od x____mo

NON-ARV PRESCRIPTION

Septrin/Dapsone _____:_____mg_____x_____days
Isoniazid _____:_____mg_____x_____days
Vit B6 _____:_____mg_____x_____days
Other _____:_____mg_____x_____days

INVESTIGATIONS None

Creatinine	Sputum	HBsAg
CD4 count	Chest X-ray	Viral load
Hb/hematocrit	CSF m/c/s	Malaria MPS
Full blood count	Pregnancy	Random Blood Sugar
ALT	RPR	Other:_e.g fPUFA_____

ADULT ARV PRESCRIPTION

FIRST LINE. Circle od or bd where applicable

(TDF + XTC + EFV) od | (TDF + XTC) od + (LPV-r) bd

(TDF + XTC) od + NVP od / bd

ABC bd + EFV od + 3TC_____ Other

OTHER FIRST LINE. Only in consultation with Medical Officer

_____ + _____ + _____ + _____

SECOND LINE. Only in consultation with Medical Officer

(AZT + 3TC + LPV-r) bd | (AZT + 3TC) bd + (ATV-r) od

OTHER SECOND LINE. Only in consultation with ATC

_____ + _____ + _____ + _____

REFERRALS None

Family planning | CaCx Screening
Nutrition supporter | Adherence counseling
Inpatient care:_____ | Community health worker
TB DOT treatment | Consented to HBC
Other:_____

ARVs dispensed for: 1 wk | 2 wks | 3 wks | 1 mo | 2 mos | 3 mos

Next clinical appointment should be in: 2 wks | 3 wks | 1 mo | 3 mos | 6 mos | Other:_____

Next pharmacy appointment should be in: 1 wk | 2 wks | 1 mo | 2 mos | 3 mos

Date of next visit: Day / Month / Year

MOH/NTH ART CLINICAL FORM

Staff Name _____ Signature _____

Appendix III

Laboratory Review Form

	NDOLA TEACHING HOSPITAL PATHOLOGY LABORATORY		
	Document Title: CD4 COUNT AND VIRAL LOAD SAMPLE REQUEST FORM		
	Document No.	Effective Date:	
	Version 3		
		Control Copy No. 0	

PATIENT'S DETAILS				
PATIENTS SURNAME:	PATIENTS FILE NO.			
OTHER NAMES:	WARD/HOSPITAL		DATE	
AGE:	SEX:			
CLINICIANS DETIALS:				
CLINICIAN'S NAME:	SIGNATURE:		TEL No.	
SAMPLE DETAILS				
SAMPLE TYPE:	DATE COLLECTED: TIME COLLECTED:			
CLINICAL DETAILS:				
FOR LAB USE ONLY RECEIVED BY(NAME)_____DATE:_____ TIME:_____ SAMPLE QUALITY: SUITABLE UNSUITABLE (EXPLAIN)_____ VIRAL LOAD RESULTS:_____ COPIES/µL CD4 COUNT_____CELLS/µL				
LAB COMMENTS: REPORTED BY(NAME)_____ SIGNATURE_____DATE:_____TIME:____ CHECKED AND AUTHORIZED BY(NAME)_____SIGNATURE:_____DATE_____TIME_ REPORT STATUS: ORIGINAL CORRECTED INTERIM FINAL				

Appendix III

	NDOLA TEACHING HOSPITAL PATHOLOGY LABORATORY **POSTAL AGENCY, NDOLA, ZAMBIA: Tel: +260212612591/3**		
	DOCUMENT TITLE: MISCELLANEOUS SAMPLE REQUEST FORM		
	DOCUMENT NO.	VERSION NO: 0	EFFECTIVE DATE:

Urgent ☐ Routine ☐

PATIENT'S DETAILS				
PATIENTS SURNAME:	PATIENT'S FILE NUMBER			
OTHER NAMES:	WARD/CLINIC		DATE	
AGE:	SEX:			
CLINICIAN'S DETAILS				
CLINICIAN'S NAME:	SIGNATURE:	TEL No:		
SAMPLE DETAILS				
SAMPLE TYPE:				
DATE COLLECTED:	TIME COLLECTED:			
INVESTIGATION(S) REQUIRED				
PATIENT CLINICAL DETAILS				

FOR LABORATORY USE ONLY

RECEIVED BY (NAME) : _____ _____DATE:_____TIME:_____

☐Suitable ☐Unsuitable (Explain_____

LAB COMMENTS:

Reported by (NAME): _____ _____ Signature_____ DATE: _____TIME: _____

Checked and Authorized by(NAME)_____ Signature_____ DATE: _____TIME:_____

REPORT STATUS:☐Original ☐Corrected ☐Interim ☐Final

Appendix IV

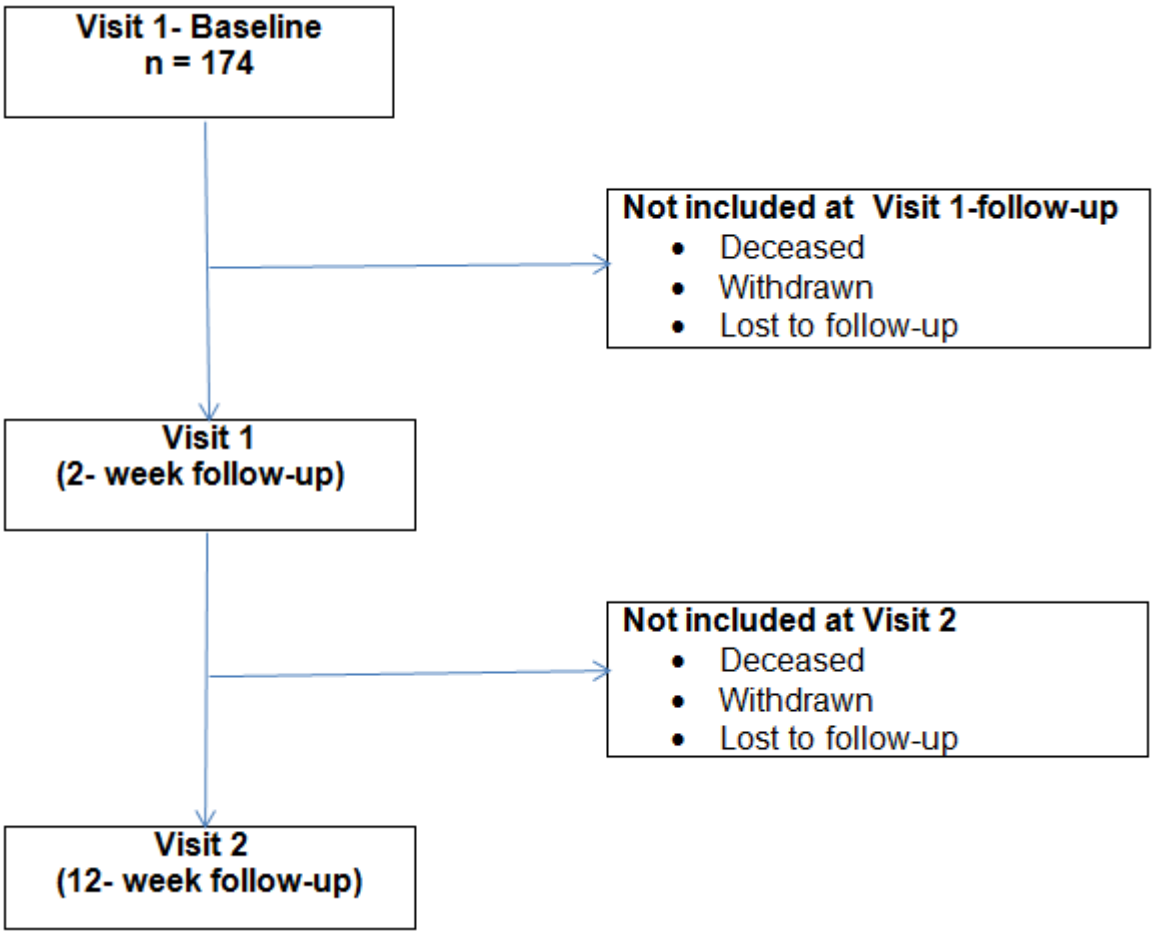
Tables and figures

Table 4.53 CHECK LIST

	TIME LINE		
ACTIVITY	**Visit 1-Baseline**	**Visit 1-follow-up**	**Visit 2**
History	√	√	√
Physical exam	√	√	√
Anthropometry	√		
Prescriptions	√	√	√
Informed consent	√		
Blood samples			
Hematology	√ **sd**	√ **rr**	
Biochemistry	√ **sd**	√ **rr**	
Serology	√ **sd**	√ **rr**	
Plasma fat	√ **sd**		√ **sd**
CD4+ count	√ **sd**	√ **rr**	√ **sd**
Viral load	√ **sd**	√ **rr**	√ **sd**

NB: sd = sample draw, **rr =** review with results

Figure 4.54. Flow diagram depicting study visits



Response Rates Table

Number of participants recruited during the Study period:	174
Number of participants in the follow-up phase	146
Study Response Rate based on total sample size*	100%
Study Response Rate based on total screened*	84%

Figure 4.55 Study procedures

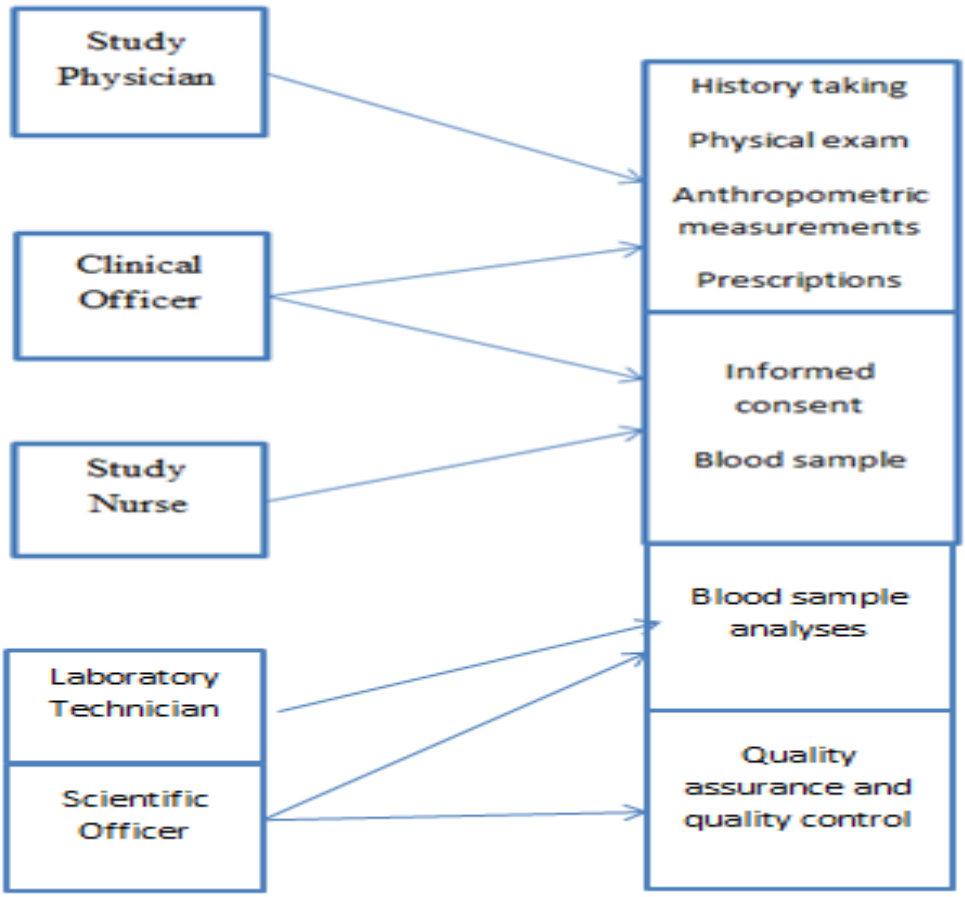


Table 4.73: Variables categorized:

INDEPENDENT	DEPENDENT	POTENTIAL CONFOUNDERS
Total Cholesterol	CD4+ count	Age
Triglyceride	Viral load	Sex
LDL-Cholesterol	Clinical status	Smoke status
HDL-Cholesterol		Alcohol status
BMI		

Table 4.74: Data description and coding table

Label	Variable	Code
Sex	Male or Female	0=male, 1= female
Opportunistic Infections	O.I [OpIn]	1= present, 0=absent
Co-morbidity	CM [CoMo]	1= present, 0=absent
Smoke status	SS [SmoSt]	1= smoker, 0= non smoker
Alcohol status	AS [AlSt]	1=alcoholic, 0=non alcoholic
Visit	V1 or V2	1= visit 1, 2=visit 2
NB: visit 2 variables to read var2		

Appendix V

Ethics approvals

All correspondence should be addressed to the
Senior Medical Superintendent
Ndola Teaching Hospital
Postal Agency
NDOLA

Telephone: 611585-9
Fax: 612204
E-mail: ***ndolateachinghospital@gmail.com***

REPUBLIC OF ZAMBIA
MINISTRY OF HEALTH
NDOLA TEACHING HOSPITAL

27th April, 2018

Dear Dr. Nyrienda C.

RE: AUTHORITY TO CONDUCT A STUDY: DR NYIRENDA C

The above subject matter refers.

I wish to inform you that we have granted you permission to conduct a research project entitled, **"Plasma fatty acids in HIV/AIDS among Zambian adults presenting to HIV Clinic at Ndola Teaching Hospital".**

Ndola Teaching Hospital embraces research activities from students and members of staff. Research enhances informed decision making at any organization. Hence, management demands that the findings of any research conducted at the hospital is shared. Information collected belongs to Ndola Teaching Hospital and permission should be sought from the institution before any publishing is done, and written authority will be granted.

The student is mandated to submit the research proposal and the final report to Ndola Teaching Hospital Research Committee through the Planning department.

The student is hereby advised to uphold confidentiality in the chosen topic of study.

Yours faithfully
Ndola Teaching Hospital

Dr. Simon Mukosai
A/Head Clinical Care
FOR/ SENIOR MEDICAL SUPERINTENDENT

TROPICAL DISEASES RESEARCH CENTRE
Tel/Fax 612837
P O Box 71769
NDOLA, ZAMBIA

TDRC ETHICS REVIEW COMMITTEE
IRB REGISTRATION NUMBER: 00002911
FWA NUMBER: 00003729

13th January 2020

OUR Ref: TRC/C4/01/2020
Dr. Christopher Nyirenda
Ndola Teaching Hospital

Ndola

Dear Dr. Nyirenda,

RE: ETHICAL APPROVAL OF STUDY PROTOCOL

Reference is being made to your letter in which you reported the amendments made to a project proposal titled **"A Study Relating Polyunsaturated Fatty Acids to Immunological Status and Viral Load in Zambian Adults with HIV/AIDS".**

On behalf of the Chairperson of the TDRC Ethics Review Committee (ERC), I wish to inform you that the Committee reviewed the responses to the queries it earlier raised and is satisfied with the responses and amendments to the protocol. Therefore, ethical approval has been granted based on the following conditions;

You are now required to submit your protocol to the National Health Research Authority for final approval following the link: **https://www.nhra.org.zm** and submit a final report to the Ethics Review Committee Secretariat at the end of the study.

This approval is valid for the period **13th January 2020** to **13th January 2021**

The Committee wishes you success in the execution of your duties.

Yours Sincerely,
TROPICAL DISEASES RESEARCH CENTRE

Edna Mwale Simbayi
SECRETARY - TDRC Ethical Review Committee

cc: Chairman - TDRC Ethical Review Committee

TROPICAL DISEASES RESEARCH CENTRE
ETHICS REVIEW COMMITTEE
13 JAN 2020
P.O. BOX 71769
NDOLA, ZAMBIA

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RESEARCH CENTRE
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Ndola,
ZAMBIA

TDRC ETHICS REVIEW COMMITTEE
IRB REGISTRATION NUMBER : 00002911
FWA NUMBER : 00003729

TRC/C4/04/2021

29th April 2021

Dr. Christopher Nyirenda
Principal Investigator
School of Medicine, Copperbelt University
Ndola

Dear Dr. Nyirenda,

RE: APPLICATION FOR AMENDMENT TO YOUR STUDY PROTOCOL

Reference is made to the study protocol entitled ***"Relating Plasma Fat to immunological, virological and clinical status in Zambian adults with HIV/AIDS"***, which was submitted for the following amendment:

- Relating Plasma **Polyunsaturated Fatty Acids** to immunological, virological and clinical status in Zambian adults with HIV/AIDS to Relating Plasma **Fat** to immunological, virological and clinical status in Zambian adults with HIV/AIDS

On behalf of the Chairperson of the TDRC Ethics Review Committee (ERC), I am pleased to inform you that your protocol was reviewed and the Committee did not have any objections to your proposed amendments.

You are further required to submit progress reports to the TDRC ERC twice a year.

Should there be any other protocol modifications or amendments, you are required to notify the ERC and submit protocol amendments for approval.

You are now required to submit your protocol to the National Health Research Authority for final approval following the link: https://www.nhra.org.zm. A final report of the study should be submitted to the Ethics Review Committee Secretariat at the end of the study.

This approval is valid for the period 29th April 2021 to 29th April 2022.

The Committee wishes you success in the execution of the study.

Yours faithfully,
TROPICAL DISEASES RESEARCH CENTRE

Sydney Mwanza
DEPUTY SECRETARY – TDRC Ethics Review Committee

TROPICAL DISEASES RESEARCH CENTRE
ETHICS REVIEW COMMITTEE
29 APR 2021
P.O. BOX 71769
NDOLA, ZAMBIA

NATIONAL HEALTH RESEARCH AUTHORITY
Paediatric Centre of Excellence, University Teaching Hospital, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@gmail.com | www.nhra.org.zm

Ref No:..... **Date: 1st June, 2020**

The Principal Investigator
Dr. Christopher Nyirenda,
Ndola Teaching Hospital,
NDOLA

Dear Dr. Nyirenda,

Re: Request for Authority to Conduct Research

The National Health Research Ethics Board (NHREB) is in receipt of your request for authority to conduct research titled "**Relating Plasma Polyunsaturated Fatty Acids to Immunological, Virological and Clinical in Zambian adults with HIV AIDS.**"

I wish to inform you that following submission of your request to the Board, its review of the same and in view of the ethical clearance, this study has been **approved** on condition that:

1. **A Material Transfer Agreement is obtained and cleared by the National Health Research Ethics Board should there be any need for samples to be sent outside the country for analysis.**
2. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised;
3. Progress updates are provided to NHRA quarterly from the date of commencement of the study;
4. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country;
5. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, and all key respondents.

Yours sincerely,

Prof. Patrick Musonda
Chairperson
National Health Research Ethics Board

NATIONAL HEALTH RESEARCH AUTHORITY
Paediatric Centre of Excellence, University Teaching Hospital, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@gmail.com | www.nhra.org.zm

Ref No: NHRA00003/13/05/2021 **Date: 13th May, 2021**

The Principal Investigator,
Dr. Christopher Nyirenda,
Ndola Teaching Hospital,
NDOLA

Dear Dr. Nyirenda,

Re: Protocol Amendment for the Study: "Relating Plasma Polyunsaturated Fatty Acids to Immunological, Virological and Clinical in Zambian adults with HIV AIDS"

Reference is made to the above subject matter.

The National Health Research Authority is in receipt of your submission of updated informed consent forms and related documents, dated April 30, 2021, for approval.

I wish to inform you that following submission of your request to the Authority, its review of the same and in view of the ethical clearance, your request has been **granted**.

Yours sincerely,

Prof. Godfrey Biemba
Director/CEO
National Health Research Authority

Appendix VI

Work schedule

No.	Activity	Responsible person	Duration
1.	Research Proposal development	Principal Investigator	3 months
2.	Research Ethics Approval	Principal Investigator/	1 month
3.	Data Collection	Principal Investigator Clinical Officer Study Nurse	1 year
4.	Data analysis and interpretation	Principal Investigator and Biostatistician	4 months
5.	Dissertation write-up/ reviews	Principal Investigator/ Supervisors	4 months
6.	Finalize dissertation Write-up	Principal Investigator	2 months
7.	Defense and submission of final dissertation	Principal Investigator	Dates to be announced
8.	Manuscript write-ups/reviews and publication	Principal Investigator/ Supervisors	5 months

Appendix VII

Budget plan

Personnel	**Units (months)**	**Unit cost per Person-kwacha (k)**	**Total personnel Cost (k)**	**Total expenses kwacha**	**Total personnel Cost US dollar ($)**	**Total expenses US dollar ($)**
Study Physician	12	5000	60,000		6000	
Medical Officer	08	2500	20 000		2400	
Scientific Officer	08	2000	16 000			
Lab Technologist	08	2000	16 000		4800	
Study Nurse	08	2000	16 000		2400	
Biostatistician	05	2500	12 500		1000	
			140,500	**140, 500**	**16, 600**	**16, 600**
Research Equipment	**Units**	**Unit cost (k)**	**Total cost (k)**		**Total cost ($)**	
Weighing scale	2	150	300		30	
BP machine	1	500	500		50	
Glucometer	1	500	500		50	
Glucostix bottles(50strips in each)	6	150	900		90	
Thermometers	5	60	300		30	
Specimen bottles	600	4	2400		240	
Tape measure	4	10	40		4	
Strapping (rolls)	6	60	360		36	
Laboratory Tests						
CD4 count	400	65	26 000		2 600	
Viral load	400	260	104 000		12 000	
plasma fat	400	N/A	46 000		3 000	
ICT Equipment/services						
Hp Laptop Computer	1	6500	6500		650	
Hp LaserJet Printer	1	4000	4000		400	
Toner for Printer	2	800	1600		160	
8GB flush	1	100	100		10	
Mobile Internet/month	12	150	1800		180	
Airtime/month	12	500	6000		600	
Stationery/Accessories						
Pens	40	5	200		20	
Markers	10	25	250		25	
Box files	5	20	100		10	
Reams of paper	10	65	650		65	
Hole punch	1	500	500		50	
Stapler	1	100	100		10	

Lockable filling Cabinet	1	2000	2000		200	
Other Expenses						
Research ethics fee	1	500	500		50	
Training study personnel	2	646	1292		129.20	
(days)						
Participant transport fee	200	20	4000		400	
(1 visit)						
Dissemination of findings						
Travel to Lusaka (fuel)	1	1000	1000		100	
Subsistence allowance	3	1000	3000		300	
Manuscript/Journal publication						
Printing and binding	1	1000	1000		100	
Journal application fees	2	5000	10 000		500	
Sub-total expense			225, 892	**225, 892**	**22, 089.20**	**22, 089.20**
Total Budget Expense				**K366, 392**		**$38, 689.20**