ANTIOXIDANT MICRONUTRIENTS INTAKE IN PEOPLE LIVING WITH HIV: IMPLICATIONS ON SERUM LEVELS AND LIVER FUNCTION

BY

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OCTOBER, 2017

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A THESIS PRESENTED TO THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF PHILOSOPHY (MPHIL) DEGREE IN HUMAN NUTRITION AND DIETETICS

BY

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OCTOBER, 2017

DECLARATION

I, Daniel Edem Kpewou hereby declare that, this thesis is my own work, carried out under the supervision of Mrs. Faustina O. Mensah and Mr. Collins A. Appiah (RD). This thesis is original and has not been presented anywhere, be it at a university or college for the award of any other degree. References cited in the text have been duly acknowledged.

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DEDICATION

This work is dedicated to God, and to my mother, Dr. Mrs. Lucy Dillys Agbozo Kpewou. Mama, you made me!!

ABSTRACT

The Human Immunodeficiency virus (HIV) infection and antiretroviral (ARV) drugs are known to cause oxidative stress which has the tendency to cause damage to body organs such as the liver, affecting their functions. Antioxidants are important to prevent oxidative stress or mitigate it. Even though some of these antioxidants can be acquired from the diet, there is insufficient data about their intakes among PLWH in Ghana. This study therefore sought to assess the intakes of these antioxidant nutrients and the serum levels of two of them, vitamin E and zinc and their possible effect on liver function of people living with HIV (PLWH) attending Antiretroviral Therapy (ART) clinic at the Volta Regional Hospital in Ho. To achieve this, 103 HIV infected adults on antiretroviral therapy were randomly sampled from a list of possible participants. A 3-day 24hr recall and a food frequency questionnaire were employed to assess vitamins A, C, E and zinc as well as energy, carbohydrates, fats and proteins intakes. Serum levels of vitamin E and zinc as well as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured. Weight and height were measured and BMI calculated. Pre HAART levels of AST and ALT as well as ARV drug intake history were also acquired from hospital records. The results showed that participants had lower median caloric (1680Kcal) and fat (54g) intakes. Median daily dietary intakes of vitamins C, D and E were 54mg, 2µg and 3mg respectively and were lower than recommended intake levels. Serum vitamin E deficiency was observed to be high among the participants (82.5%). The prevalence of underweight, overweight and obesity in the study were 11.7%, 21.4% and 11.7% respectively. There was a significant rise in serum AST levels, from 22.0 IU/L pre HAART to 30.4 IU/L post HAART initiation. Serum levels of ALT significantly decreased from 17.0 IU/L pre-HAART to 13.0 IU/L post-HAART. There was no association between the serum levels of vitamin E and zinc and serum AST and ALT levels. The findings from this study suggest that, serum levels of antioxidant micronutrients, vitamin E and zinc, did not have any effect on liver function.

ACKNOWLEDGEMENT

Glory to God now and always for all He has done and continues to do for me. My profound gratitude goes to my supervisors Mrs. Faustina O. Mensah and Mr. Collins Afriyie Appiah, RD, for their guidance, dedication patience and efforts that led to the success of this work.

I wish to express my sincere thanks to the Medical Director and staff of the Volta Regional Hospital most notably to Dr. Emmanuel Kasu, Madam Akpene Awittor and Madam Emma Akubia of the Anteretrovirial Therapy Clinic, as well as Mr. Maxwell Gbemu of the Laboratory. I also thank all the participants who voluntarily took part in this study.

I specially extend my gratitude to Dr. Reginald A. Annan for his mentorship, encouragement, wise counsel and godly inspirations throughout the period of study. This acknowledgement will be incomplete if I don't mention Mr. Ernest Doe and Professor Senyo Adjibolosoo, both of the USA. Thank you both for believing in me. I also wish to thank Dr. Huseini Wiisibie Alidu for his advice during the course of this work.

I extend my heartfelt appreciation to my parents and entire family as well as friends for their support in diverse ways. Many people, in diverse ways contributed to the success of this work. Even though the list is long, I remember and acknowledge them all.

God bless you all.

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LIST OF ABBREVIATIONS

AIDS	-	Acquired Immune Deficiency Syndrome
ALT	-	Alanine Aminotransferase
ARV	-	Antiretroviral drug
AST	-	Aspartate Aminotransferase
BMI	-	Body Mass Index
CHRPE	-	Committee on Human Research, Publications and Ethics
DNA	-	Deoxyribonucleic Acid
FIV	-	Feline Immunodeficiency Virus
GDHS	-	Ghana Demographic Health Survey
GHS	-	Ghana Health Service
HAART	-	High Active Antiretroviral Therapy
HIV	-	Human Immunodeficiency Virus
HPLC	-	High Performance Liquid Chromatography
HTLV	-	Human T cell Lymphotropic Virus
LAV	-	Lymphadenopathy Associated Virus
NNRTI	-	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	-	Nucleoside Reverse Transcriptase Inhibitors
OS	-	Oxidative Stress
PI	-	Protease Inhibitors
PLWH	-	People Living With HIV
PLWHA	-	People Living With HIV and AIDS
RDA	-	Recommended Dietary Allowance
RNA	-	Ribonucleic Acid
RNS	-	Reactive Nitrogen Species

ROS	-	Reactive Oxygen Species
SD	-	Standard Deviation
SIV	-	Simian Immunodeficiency Virus
SOD	-	Superoxide Dismutase
SPSS	-	Statistical Package for Social Science
WHO	-	World Health Organization

CHAPTER ONE

1.1 INTRODUCTION

According to the UNAIDS (2006), there were 2.1 million new HIV infections worldwide in 2015 which increased the total number of individuals living with the virus to 36.7 million. The Ghana AIDS Commission (GAC) stated that as at 2014, there were 250,232 people living with HIV (PLWH) in Ghana (Ghana AIDS Commission, 2015) as compared to 195,077 in 2011(UNAIDS, 2011). Out of the 2014 figure, 92% were adults of which 59% and 41% were females and males respectively with a national adult HIV prevalence of 1.47%. The estimated incidence of HIV among adults in Ghana as at 2014 was 0.07%, comprising 11,356 and 9,248 new infections and AIDS-related deaths respectively (Ghana AIDS Commission, 2015).

Studies have shown that HIV infected individuals experience oxidative stress given that the virus and the antiretroviral drugs are both known to produce free radicals that cause this oxidative stress (Akay *et al*., 2014; Touzet and Philips, 2010; Obirikorang, 2009). With reported low serum antioxidant levels (Allard *et al*., 1998) and micronutrient deficiencies (Stephensen *et al*., 2006), PLWHs stand a risk of experiencing the deleterious effects of oxidative stress such as cancers, liver damage, heart diseases, diabetes and other degenerative conditions. Moreover, antiretroviral drug use has also been shown to raise transaminases levels and this coupled with increased oxidative stress, may lead to liver damage (Núnez, 2006).

While other studies have investigated the levels of oxidants and their effects on HIV positive individuals, the effect of antioxidant micronutrients (vitamin E and zinc) on liver function among these HIV positive people remain to be elucidated. The present study therefore sought to determine the possible effect of dietary intake on serum levels

of specific antioxidant micronutrients (vitamin E and zinc) and liver function among PLWH.

1.2 PROBLEM STATEMENT

The Ghana AIDS Commission stated that in 2014, there were 9,248 deaths caused by AIDS (Ghana AIDS Commission, 2015). No cure has yet been found for HIV and AIDS (UNAIDS, 2003). It has been reported that disease (Allard *et al*., 1998) and antiretroviral (ARV) drugs (Akay *et al*., 2014; Touzet and Philips, 2010; Mandas *et al*., 2009) cause oxidative stress. Oxidative stress in HIV patients is the major cause of death (Valle *et al*., 2013; Mandas *et al*., 2009; Núnez, 2006).

A study by Obirikorang (2009) among PLWH in the Bolgatanga and Central Regional Hospitals in Ghana revealed that there is increase in oxidative stress as HIV progresses. There is paucity of data on antioxidant nutrient intake in PLWH in Ghana and the effect on serum levels and liver function.

1.3 RESEARCH QUESTIONS

This research sought to answer the following questions:

- What are the usual diets consumed by HIV infected individuals and the antioxidant nutrient composition of these diets?
- What are the serum levels of vitamin E and zinc among PLWH?
- What is the relationship between levels of antioxidant nutrient intake, serum levels and liver function?

1.4 MAIN OBJECTIVE

The main objective of this study was to assess the antioxidant micronutrient intake, serum levels and relationship with liver function among HIV positive individuals undergoing antiretroviral therapy at the outpatient department of the Volta Regional Hospital in Ho.

1.4.1 Specific objectives

- To assess the dietary intake of antioxidant micronutrients by PLWH at the outpatient department of the Volta Regional Hospital in Ho.
- To determine specific serum antioxidant micronutrients (vitamin E and zinc) levels among PLWH at the outpatient department of the Volta Regional Hospital in Ho.
- To assess alanine transaminase (ALT) and serum aspartate transaminase (AST) status of the study participants.
- To determine the relationship between level of intake, serum antioxidant micronutrients (vitamin E and zinc) levels and liver function among PLWH.

1.5 JUSTIFICATION

Deficiencies in vitamins and minerals are common in HIV infection (Garcia-Prats *et al*., 2010). Besides, chronic immune activation and increased oxidative stress in PLWH may lead to increased nutrient needs beyond the recommended intakes (Stephensen *et al*., 2006; Jahoor *et al*., 2003). Prolonged use of HAART has also been suggested to cause oxidative stress in PLWH (Núnez, 2006). The liver is the major organ affected by oxidative stress (Sánchez-Valle *et al*., 2012). Even though research by Baum *et al*., (2006) indicated that, micronutrient supplementation can significantly improve CD4+

cell count reconstitution in HIV-infected patients undergoing HAART, there is less information about the intake of antioxidant micronutrient containing foods, their serum levels, and possible effect on liver function among PLWH in Ghana.

Through this research, PLWHs, their carers and other stakeholders will be equipped with knowledge regarding the intakes of foods containing these antioxidant micronutrients among the PLWH. This will help in achieving behavioural change or reinforcement towards consumption of these foods. Furthermore, results of the study will generate knowledge which will be applied by nutritionists, dietitians, and other healthcare givers in the management of PLWH through the development and or revision of policies and management protocols.

CHAPTER TWO

LITERATURE REVIEW

2.1 BACKGROUND OF HIV/AIDS

In 1981, a new syndrome, the Acquired Immune Deficiency Syndrome (AIDS) was first recognised in the United States among gay men. It was not until in 1983, when the causative agent, human immune deficiency virus (HIV) was identified. By this time however, the infection had spread throughout many parts of the world, mostly unnoticed (Maartens *et al*, 2014; De Cock *et al*., 2011).

Suggestions have it that, HIV originated from non-human primates in Sub-Sahara Africa and was transmitted to human between the late19th and early 20th centuries. This is based on the fact that, the virus has a close similarity to another one, the simian immunodeficiency virus (SIV) which is found in chimpanzees in West-Central Africa and the Sooty Mangabey (white-collared monkey), native to West Africa (Sharp *et al*., 2001).

Even though the precise time of the occurrence of the zoonosis is unknown, some researchers proposed that it happened between 1915 and 1941 (Korber *et al*., 2000). There have been many suggestions about the basis of AIDS. Some proposed that it was not caused by HIV and others totally denied the existence of HIV or AIDS. Some people believe lifestyle including sexual activity and drug use are the causes of AIDS. Others alleged that HIV was created in a bio-weapons laboratory as an agent of genocide or accident. The mainstream scientific community however, based on scientific consensus has rejected these claims (Adum *et al*., 2015; Smith and Novella, 2007; Maggiore, 2000) and concluded that AIDS is caused by HIV which is transmitted through blood and other body fluids via some specific routes.

2.2 EPIDEMIOLOGY OF HIV INFECTION

2.2.1 Global

According to the UNAIDS report of 2016, an estimated 36.7 million (34.0 million–39.8 million) people were living with HIV worldwide in 2015. This figure is 10% higher than the prevalence of HIV in 2010 and about 18% more than the prevalence in 2005. The estimated number of new HIV infections globally in 2015 was 2.1 million (1.8 million–2.4 million). While an estimated 1.1 million (940, 000 –1.3 million) AIDS related deaths were realised in the same year, a 26% decline from an estimated 1.5 million (1.3 million–1.7 million) AIDS-related deaths globally were recorded since 2010. Epidemiological data suggests that reduction in new adult HIV infections have declined alarmingly in recent years, with the estimated yearly number of new infections among adults remaining nearly stagnant at around 1.9 million (1.7 million–2.2 million) in 2015 (UNAIDS, 2016).

There are however multiple differences across regions, within countries, between genders and age groups and among specific populations being left behind. Eastern and Southern Africa saw the biggest decrease in HIV infections among adults; a 4% decline in 2015 compared to 2010, while, Western and Central Africa, Asia and Pacific region had more gradual declines. While the Caribbean and Latin America, Western and Central Europe, the Middle East and North Africa and North America recorded relatively static rates of new HIV infections among adults, there was increase by 57%, the yearly numbers of new HIV infections in Eastern Europe and Central Asia. The prevalence of HIV in cities is higher than that of rural areas in many countries. Among adults globally, young women aged 15–24 years and adolescent girls are particularly at higher risk of HIV infection, forming 20% of new HIV infections in 2015, even though they account for just 11% of the adult population. According to current reports, sex

workers, homosexual men, transgender people, people who inject drugs, and prisoners and are still key populations at an increased risk of being infected with HIV (UNAIDS, 2016).

2.2.2 Ghana

In Ghana, the first case of HIV/AIDS was identified in 1986. By December 2000 however, about 330,000 and 20,000 adults and children respectively were thought to be already infected with the virus, with the health ministry reporting 43,587 AIDS cases. The epidemic which has since spread slowly but steadily reached a national adult prevalence of 3.0% in the year 2000 from a prevalence of 2.7% in 1994. The UNAIDS (2011) stated that there was a 39% decline in the prevalence of the disease among antenatal clinic attendees in Ghana from the years 2001 through 2010. In 2013, an estimated 224,488 people were living with HIV with an estimated prevalence rate of 1.3%. In the same year, there were 7,812 new infections and 10,074 deaths caused by AIDS (Ghana AIDS Commission, 2015). The 2014 national prevalence of HIV is 1.47% comprising 11,356 and 9,248 new infections and AIDS-related deaths, respectively (Ghana AIDS Commission, 2015).

Even though data indicated that HIV infection exists in all parts of the country, the prevalence rates are not uniform across the regions. The highest prevalence was recorded in the Eastern region while Northern region recorded the lowest. Urban areas, towns along major roads, densely populated areas, mining and border towns have high prevalence. The male to female ratio among the HIV population is 1:1.5 with approximately 59% of infected individuals being females. The prevalence of HIV among urban area dwelling pregnant women has been consistently higher than the prevalence of their rural area dwelling counterparts. The HIV prevalence among

pregnant women age 35-39 years was 3.2% as compared with 0.9% among the 15-19 year group in 2014 (Ghana AIDS Commission, 2015).

2.3 CLASSIFICATION OF THE HIV

The human immunodeficiency virus (HIV) belongs to the viral family *retroviridae* and a genus *lentivirus* of which includes its closest relatives the feline immunodeficiency virus (FIV) and the simian immunodeficiency virus (SIV) (Sierra *et al*., 2005). The FIV causes diseases in cats and SIV causes diseases in monkeys. Ribonucleic acid (RNA) is the form in which genetic information of retroviruses is stored. The retroviruses use reverse transcriptase, an enzyme to reverse-transcribe their RNA to deoxyribonucleic acid (DNA) once they infect a host cell. The replication of HIV is rapid with many mutations causing high variability in strains. Due to this, there are many viral strains that have been classified into types, groups and subtypes (Plantier *et al*., 2009; Sierra *et al*., 2005).

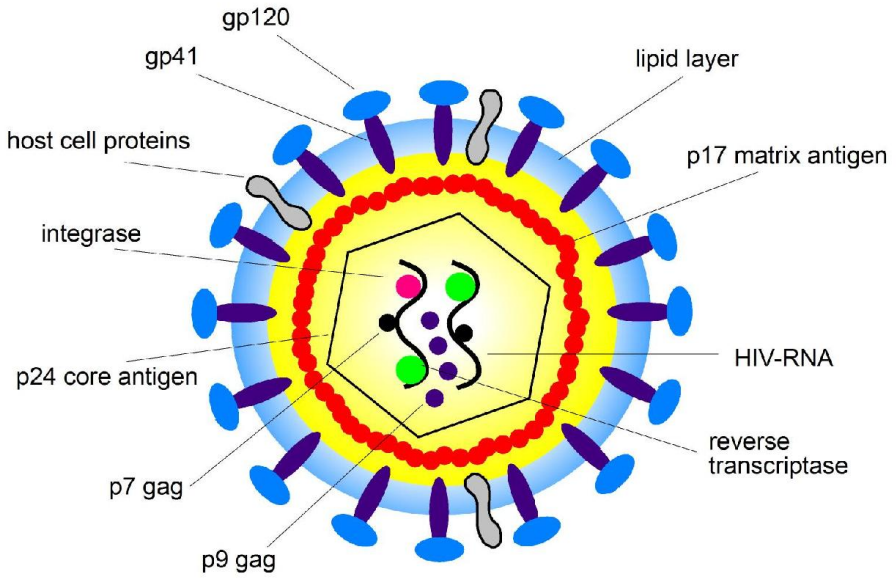
2.3.1 Types of Human Immunodeficiency Virus

Currently, there are two main types of HIV. These are HIV-1 and HIV-2 which are both transmitted through sexual contact, needle sharing and from mother to child during pregnancy, delivery or breastfeeding (Plantier *et al*., 2009). They are also similar in intracellular mechanisms of replication and clinical results. HIV-1 is considered to originate from the simian immunodeficiency virus, SIV_{cpz} strain that infects chimpanzees (Heeney *et al*., 2006) with HIV-2, originating from the SIV_{sm} strain that is carried by the sooty mangabey (White collared monkey) (Visseaux *et al*., 2016). The rate of transmission is slower for HIV-2 compared to HIV-1 and progression to AIDS is less frequent or slow in HIV-2 compared to a more virulent

HIV-1 (Reeves *et al*., 2002). Both types of viruses can be found in one individual and is known as dual infection. While HIV-1 is found globally, HIV-2 is hardly identified elsewhere as it has been mostly endemic in West Africa (Reeves *et al*., 2002).

2.4 BIOLOGICAL STRUCTURE OF HIV

Just like all other viruses, HIV is a non-living particle when outside a living cell and thus cannot grow or reproduce on its own. It therefore needs to infect a living cell to be able to make new copies of itself. Each HIV particle usually called a virion is crudely round and has a diameter of around 100nm to 120nm. Each virion is covered with a lipid bilayer, the viral envelope, which is acquired from the membrane of the host cell during replication as the virus buds out of the host cell. Studded on the envelope are peg-like structures formed from the proteins gp120 and gp41. There are about three to four gp41 proteins and three to four gp120 proteins forming the stem and cap respectively, of the peg. Under the envelope is a shell made from the p17 protein which surrounds a conical core or capsid, made from p24 protein. The capsid contains two identical single strands of RNA encapsulated in nucleocapsid (p7) stabilised together as a ribonucleoprotein. The capsid also contains three enzymes integrase, protease and reverse transcriptase, which are vital to the life of the virus (Sundquist and Kräusslich, 2012; Orenstein, 2002).



Source: Adapted from HIV2015/16 by Christian Hoffman and Jürgen K. Rockstroh. Retrieved from www.HIVbook.com

Figure 2.1: Structure of HIV

2.4.1 Genes of HIV

HIV has only nine genes contrasting about 25,000 genes in its human host. All the genes are located on one long strand of RNA. Even though in a free virus, there are actually two separate strands of RNA they are essentially exactly the same (Two of the same RNA copies exist at a time in a free virus). The genes include *env*, *gag*, and *pol* genes that code for specific structural proteins, such as the viral capsid and envelope associated proteins. The others *rev*, *tat*, *vif*, *nef*, *vpr*, *vpu* (not found in HIV-2) and *vpx* (not found in HIV-1), are regulatory genes which code for proteins that control the infective, reproductive and pathogenic abilities (Levy, 2009; Sierra *et al*., 2005).

2.4.2 The life cycle of HIV

2.4.2.1 Attachment and entry

For HIV to replicate, it needs to enter a human host cell. The virus only infects certain cell types. Although the virus mainly targets the helper T lymphocytes (T_4 or CD4+),

other cells such as macrophages can also become infected. These cells which HIV infect have on them some special proteins; CD4+, CCR5 and CXCR4 which it binds to. Infection begins when a single or numerous viral gp120 proteins bind to CD4+ receptors on the host cell. CCR5 and CXCR4 are both co-receptor molecules that have to be bound by the virus surface proteins (Sierra *et al*., 2005). This binding allows for fusion of the virus to the cell membrane of the host cell and subsequent release of viral genome and other contents into the host cell, leaving its envelope behind (Magérus-Chatinet *et al*., 2007; Stebbing *et al*., 2004; Murdoch and Finn, 2000).

2.4.2.2 Reverse transcription, migration and integration

In the cell, reverse transcriptase, an HIV enzyme, uses the single stranded viral RNA as a template to transcribe a single stranded DNA within the capsid. The single stranded DNA acts as a template in the formation of a second strand of DNA, making a double stranded DNA. The double stranded DNA is then transported to the nucleus of the cell. Once in the nucleus, the enzyme integrase, integrates this newly formed HIV DNA into the host cell's DNA, forming a provirus (Jouvenet *et al*., 2011; Moir, 2011; Campbell and Hope, 2008; Vandegraaff and Engelman, 2007).

2.4.2.3 Transcription and translation

When the cell divides, the provirus replicates alongside the chromosomes. It may however lie dormant within a cell for a period of time and these cells are called resting cells. Upon activation, the provirus employs RNA polymerase of the host cell in the generation of copies of the genomic material of HIV as well as messenger RNA. The messenger RNA is later transported out of the nucleus into cytoplasm where it is employed as a blueprint to produce long chains of viral proteins.

2.4.2.4 Assembly, budding and maturation

The long chains of viral proteins are cut into smaller ones by the HIV enzyme protease. A new HIV particle is then formed when the smaller proteins and the viral genetic material are assembled together. The newly assembled virus begins to bud out of the cell by pushing against the plasma membrane of the host cell and in the process takes portion of the host cell's plasma membrane which serves as the viral cover. Inserted in the viral cover are HIV glycoproteins which are important for the virus in the binding to receptors and co-receptors of host cells.

The new copies of HIV are now mature and can now move to infect other cells. It should be noted that an HIV infected cell does not break down immediately after viral replication has occurred. Many more viral particles may bud out of that same cell over a period of time, making the host cell to act like a "virus producing factory" (Sundquist and Kräusslich, 2012; Briggs and Kräusslich, 2011).

2.5 TRANSMISSION OF HIV

The virus is primarily found in body fluids such as blood and blood components, semen, breast milk, and vaginal secretions of infected people. It is thus transmitted through these fluids in three different ways;

- Unprotected sexual intercourse (vaginal, anal or oral) with someone infected with HIV
- Direct injection with HIV-contaminated drugs, needles, syringes, blood or blood products, and
- From HIV-infected mothers to foetus in utero, through inoculation from mother to infant during birth, or during breast-feeding.

The HIV is delicate, it is unable to survive outside the live human system for long. As such daily activities such as a casual kiss, hugging, or handshaking cannot lead to its transmission. Infection is impossible through drinking of fountains, sharing toilet seats, drinking glasses, dishes, foods or from pets. HIV is not also transmitted through mosquito bites. It cannot be transmitted through tears, saliva, urine, perspiration, sputum, faeces or vomitus (Shepard *et al*., 2000).

Some activities pose greater risks of infection compared to others. Indulging in sexual intercourse without protection with an infected person is associated with a higher risk of infection but having multiple sexual partners increases that risk due to increased exposure (Lamptey, 2002). Unprotected vaginal sexual intercourse is less risky compared to unprotected anal sexual intercourse. It is currently known that the most efficient mode of sexual transmission is unprotected anal sex (Lamptey, 2002). As such, homosexual men have a greater risk of infection with the virus than the general population (Patel *et al*., 2014). Unprotected oral sex can contribute to HIV transmission but it is of a much lower risk than anal or vaginal sex (Patel *et al*., 2014). Other sexually transmitted diseases such as hepatitis and herpes may increase the risk of HIV infection. It has been shown that, uncircumcised men are at a greater risk of being infected with the virus than circumcised men (Royce *et al*., 1997). Sharing of syringes, needles, or other equipment used in the preparation of injectable illicit drugs are high risk sources of infection. Mother to child transmission risk during pregnancy, childbirth or during breastfeeding can be reduced with appropriate prenatal and postnatal evaluation and treatment (Thorne and Newell, 2007).

2.6 THE NATURAL COURSE OF HIV INFECTION

2.6.1 Acute HIV syndrome phase

Following initial infection with the virus, about 40% to 70% of infected individuals experience the acute stage. They exhibit symptoms similar to those of flu or mononucleosis. Some of these symptoms include headache, fever, diarrhoea, sore throat, generalised lymphadenopathy and erythematous rash. Helper T cell counts, which is determined as the number of cells per microlitre, drops after an initial rise due to the body's immune response to the infection. Normally, the ratio of CD4 to CD8 is around 2:1 but this also falls to 0.5 or below. This acute illness typically resolves spontaneously from the second to third week (Yerly and Hirschel, 2012).

Research indicates that apart from the virus passing through the blood and lymph systems during the acute phase, it also seeds in certain cells known as latent reservoirs. The virus is protected from many medications in these reservoir which serves as a hiding place (Yerly and Hirschel, 2012).

2.6.2 HIV- specific immune response

It takes from a few weeks to several months for the human body to mount a humoral response to HIV. The response is much faster with HIV compared to other pathogens. This time is referred to as the "window period" of the disease. Seroconversion occurs but only after the HIV-specific immune response has been initiated, and at this time, most diagnostic tests indicate positive results, signifying the activation of HIV antibodies (Barouch and Deeks, 2014; Sierra *et al*., 2005).

2.6.3 Clinical latency

In this phase, few or no symptoms are seen in an HIV infected person. A normal range CD4+ cell count of 800 to 1200 cells/μL may be achieved again, or levels may become steady but low, or gradually decrease. Bodily virion counts approach the set point, an equilibrium value. At the set point, the immune system is able to keep the virus from replicating uncontrollably. Despite the asymptomatic nature of this phase, HIV still remains active in the lymphatics, replicating aggressively and destroying helper T cells. The body however continues to fight the infection until the CD4+ cell population is depleted below healthy levels. The clinical latency phase can last averagely for 10 years (Barouch and Deeks, 2014; Hogan and Hammer, 2001; Vergis and Mellors, 2000).

2.6.4 AIDS-defining illnesses

At this stage of infection, viral load in individuals may be very high, about one million copies/ml, even though individual differences are significant. As CD4+ T cell counts fall below about 400 cells/μL, major clinical symptoms begin to show. Some of these include, fever, fatigue, diarrhoea, weight loss, persistent generalised lymphadenopathy and strong smelling and profuse night sweats. The body is now prone to many infections like vaginal and oral candidiasis, listeriosis, herpes zoster, herpes simplex, and oral hairy leukoplakia, which also, mostly sets in. When CD4+ T cell count falls below 200 cells/μL, other opportunistic infections such as mycobacterium tuberculosis, Kaposi's sarcoma, pneumocystis *Jirovecii pneumonia*, *Mycobacterium avium* complex, and others cause destructions of the body until one or more of them cause demise. It is the opportunistic infections that kill patients and not the virus itself. Opportunistic infections and cancers cause about 90% and 7% respectively of AIDS related deaths (Chou *et al*., 2012; Kaplan *et al*., 2009; Appay and Sauce, 2008; Sierra *et al*., 2005).

2.7 HIV AND ANTIRETROVIRAL DRUGS

Medications that are currently being used to manage HIV infected individuals are known as antiretroviral (ARV) drugs. These anti-HIV drugs have been designed to prevent key steps in viral uptake and reproduction, hindering viral proliferation and effects. These drugs achieve this by inhibiting important receptors and the enzymes that catalyse these steps in the virus's life cycle (Warnke *et al*., 2007).

The development of drugs that target and inhibit HIV enzymes; reverse transcriptase and protease, caused a revolution in the treatment of HIV-1 infection. The combination of three or more anti-HIV drugs, known as highly active antiretroviral therapy (HAART) was started in 1996. HAART has provided extraordinary benefits to HIV infected individuals and has markedly decreased morbidity and mortality among these individuals, making the infection a manageable chronic disease (Kitahata *et al*., 2009; Hammer *et al*., 2006). Furthermore, HAART has led to reduction in HIV transmission (Cohen *et al*., 2011; Reynolds *et al*., 2011). Antiretroviral drugs, in spite of their immense effect on HIV infection, present with adverse effects. Some of these adverse effects include; dyslipidaemia, diarrhoea, nausea, vomiting, drug induced hepatitis, hepatic decompensation, steatosis, severe hepatotoxicity, spontaneous bleeding and insulin resistance (Gardner *et al*., 2014).

The durability and efficient suppression of plasma viremia and the delay in or prevention of drug-resistance mutations is guaranteed by HAART. These help in the preservation or improvement of CD4+ lymphocyte cell counts which confers immense clinical gains, which are vital treatment aims (García *et al*., 2004).

2.7.1 Classes of antiretroviral drugs

These include nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTI), entry inhibitors (EI) and maturation inhibitors.

2.7.2 Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs)

These are a category of oral ARV drugs that interrupt the HIV replication cycle by competitively inhibiting HIV reverse transcriptase thereby terminating the DNA chain (Weller and Williams, 2001). This they achieve by being incorporated in the growing proviral DNA (Elion *et al*., 2008). Members of this class include tenofovir disoproxil fumarate (TDF), abacavir sulfate (ABC) stavudine (d4T), zidovudine (ZDV), emtricitabine (FTC), lamivudine (3TC), didanosine (ddI) and zalcitabine (This is no longer available commercially) (Thompson *et al*., 2012). These drugs however generate drug intolerance and toxicity problems. Most of these adverse effects seem to be mediated through mitochondrial toxicity, associated with older NNRTIs such as zidovudine, zalcitabine, stavudine and didanosine. All NRTIs may lead to gastrointestinal symptoms such as nausea and vomiting as well as headache. Zidovudine can cause severe bone marrow suppression alongside anaemia especially during the initial months of use (Gardner *et al*., 2014).

2.7.3 Non-nucleoside/nucleotide Reverse transcriptase inhibitors (NNRTIs)

Drugs in this class also interrupt viral replication just as NRTIs, but then achieve their therapeutic effects by directly binding to reverse transcriptase thereby interfering with its role in the replication process. Drugs in this class include, efavirenz (Sustiva),

delavirdine (Rescriptor), and nevirapine (Viramune). Etravirine (Intelence), and rilpivirine (Edurant) are however, second generation NNRTIs approved in the United States for use (FDA News, 2011; Warnke *et al*., 2007).

These drugs have been reported to cause hepatotoxicity with hepatic enzyme elevation. The risk of hepatotoxicity is increased when PIs are used in combination with either efavirenz or nevirapine compared to using efavirenz or nevirapine alone (Gardner *et al*., 2014).

2.7.4 Protease inhibitors (PIs)

Protease inhibitors prevent cleavage of viral precursor proteins into subunits required to form new virions by binding to the HIV protease enzyme. Drugs approved in this class include, amprenavir, ritonavir, atazanavir, fosamprenavir, darunavir, lopinavir/ritonavir, indinavir, nelfinavir, saquinavir and tipranavir. They are more effective when combined with reverse transcriptase inhibitors. All PIs especially ritonavir are known to raise hepatic transaminases levels and also cause gastrointestinal symptoms as well as metabolic abnormalities such as fat accumulation, hyperlipidaemia, peripheral insulin resistance and impaired glucose tolerance (Gardner *et al*., 2014; Wensing *et al*., 2010; Warnke *et al*., 2007; Noor *et al*., 2004).

2.7.5 Entry inhibitors (EIs)

This class of drugs block virus attachment to receptors of the cell surface membrane. They are different from other classes of ARVs because they have an extracellular mode of action. Vicriviroc and maraviroc are examples of selective CCR5 inhibitors that prevent membrane fusion. Cenicriviroc blocks both CCR5 and CCR2. A subcategory of this class of antiretroviral drugs are the fusion inhibitors that block gp41 mediated

fusion. Enfuvertide is an example of fusion inhibitor that is administered via subcutaneous injection (Haqqani and Tilton, 2013; Henrich and Kuritzkes, 2013).

2.7.6 Integrase strand transfer inhibitors (INSTI)

By the inhibition of the enzyme integrase, drugs of this class are able to prevent integration of viral RNA into the DNA of its host cell. Raltegravir and elvitegravir are examples of drugs in this class. Raltegravir is linked with myopathy as a result of skeletal muscle toxicity (Andrews and Heneine, 2015; Lee *et al*., 2013).

2.7.7 Maturation inhibitors

Maturation inhibitors prevent HIV from producing mature infectious virus particles by blocking the assembly of virions. The antiretroviral drug beviramat achieves this by inhibiting the HIV *gag* gene's function (Wang *et al*., 2015).

2.8 NUTRITION AND HIV/AIDS

A close association exists between nutrition and HIV infection (Mondy *et al*., 2002). This association has both clinical and social implications. Generally, infections, no matter their severity, influence nutritional status. If this influence causes a severe deficiency in any nutrient, there will be decline in the body's resistance to infection (Scrimshaw and SanGiovanni, 1997), leading to more infection and further deterioration of nutritional status. This relationship shows a typical instance of the well- documented "vicious cycle" of immune malfunction, infectious disease, and malnutrition (Semba and Tang, 1999).

Nutritional status is affected by infections through; reduction in dietary intake and nutrient absorption as well as increased use and excessive loss of protein and

micronutrients. The latter is a result of the mounting an "acute phase response" to invading pathogens. The acute phase response is frequently associated with anorexia, fever, and increased catabolism, all of which affect energy utilization, hence, nourishment and nutritional status are directly or indirectly affected. In their review on the effect of HIV and malnutrition on immune function, Duggal *et al*., (2012), upheld that, both HIV and malnutrition reduce immunity. They concluded that timely and serious dietary interventions should form an integral part of the treatment of PLWHA since it has the tendency to slow or even prevent the development of nutritionally acquired immune deficiency syndrome (NAIDS) and to enhance both length and quality of their lives.

Poverty and food insecurity may lead to migration and high-risk sexual behaviours which increases HIV infection risk (Weiser *et al*., 2007; Harvey, 2003; Loevinsohn and Gillespie, 2003). On the other hand, a weakened body due to HIV infection or opportunistic infections, will affect people's ability to produce food or generate income to acquire it, leading to food insecurity and subsequent nutrition insecurity. This is primarily observed among individuals living with HIV and to large extent, the whole community (Mutangadura *et al*., 1999). Fields-Gardner (2010) pointed out that, nutrition intervention has prospects in improving modifiable risk factors for metabolic disorders among PLWH and is essential in symptom management.

The main aims of nutrition intervention thus should be to optimise nutrition status, immunity and overall well-being; achieve and maintain an ideal body weight; prevent and correct any nutrient deficiencies; reduce the risk of onset of complications of co-morbidities and help in maximising effectiveness of medical and pharmacological managements. Nutrition interventions in this case may take the form of counselling on

specific behaviours, prescribed or targeted nutrition supplements, and linkages with food-based interventions and programs.

2.8.1 HIV infection and effects on nutrition

Studies have indicated that HIV infection compromises the nutritional status of infected persons therefore, a poor nutritional status has been shown to affect the progression of the infection to AIDS (Piwoz, 2004; Jiamton *et al*., 2003; Lorenz *et al*., 2001). Nutritional status impairment has been shown to exist even in the early asymptomatic phase of the disease (Bogden *et al*., 2000). There may however be more complication in the relationship between HIV and nutrition compared to that between other infectious diseases and nutrition. This is due to the direct, attack and destruction of the immune system cells by the virus (Piwoz and Preble, 2000). This immune impairment leads to malnutrition which also results in further weakening of the immune system and hence a rapid development of AIDS (Enwonwu, 2006).

An HIV infected person's nutritional status can be affected by the infection in various ways. One way by which this occurs is through the decline in food intake due to oral thrush, oesophagitis, sore throat, depression, fever, nausea and vomiting, all which lead to an ensuing anorexia (FANTA, 2004). Malabsorption of nutrients as a result of destruction of gastrointestinal tract by HIV and diarrhoea caused by bacterial infections also adversely affect the nutritional status. Increased resting energy expenditure, utilization and excretion of proteins and micronutrients, cytokine effects, hormonal disturbances and metabolic changes all lead to both macronutrient and micronutrient malnutrition in HIV infected individuals (WHO, 2004; Piwoz and Preble, 2000; Macallan, 1999).

Deficiencies in nutrients affect the normal immune function and may influence viral replication and expression which in turn affects progression to AIDS and mortality. Semba and Tang (1999) also noted that, oxidative stress may indirectly hasten HIV replication. Some micronutrients such as vitamins A, C and E as well as zinc however serves as antioxidants which offsets the harmful effects of oxidative stress.

Malnutrition is the key clinical manifestation of AIDS and a contributor to the advancement of HIV infection to AIDS (Piwoz and Preble, 2000). It can present in various forms including macronutrient and micronutrient malnutrition. In recent times however, macronutrients deficiency among PLWH which mostly present as either, wasting, underweight or stunting are less common especially among HIV positive individuals living in developed nations and has mostly been attributed to the availability of HIV treatment for majority of infected people. In a study conducted in Zimbabwe for example, it was found that less HIV patients were underweight compared to those who were overweight or obese (Takarinda *et al*., 2017). There are however still, emerging questions regarding the significance of micronutrients in HIV infection, following the introduction of HAART (Tang *et al*., 2005). Micronutrient deficiencies are frequent among PLWHAs and this reduces the ability of their immune systems to fight infections therefore contributing to rapid disease progression (Fawzi *et al*., 2002). The shift from, chronic diarrhoea, AIDS wasting syndrome and stunting to conditions associated with oxidative stress, increased inflammation and immune activation as result of chronic HIV infection are the new issues to be tackled (Dee Pee and Semba, 2010; Suttajit, 2007).

2.8.2 Interaction between Antiretroviral drugs and nutrition among HIV positive individuals

Generally, medications interact with food and nutrition. This interaction may lead to positive or negative outcomes considering the overall health and nutritional status of a person (Castleman *et al*., 2004). Drug-nutrition interactions, the overarching term used for interactions of such nature (Williams *et al*., 2010; Schwartz, 2009; Kurt *et al*., 2006), extend beyond issues of bioavailability of food or specific food components in the gut and its effects on drugs (Boullata, 2013; Raiten *et al*., 2005). Drug-nutrition interactions however come about through chemical, physical, physiological or pathophysiological associations not only between a drug and a nutrient but also between a drug and various nutrients, food in general, specific foods or components, or nutritional status (Boullata, 2010; Santos and Boullata, 2005). The interaction can be a combined or reciprocal action between any of the elements involved, where one element or group of elements acts as a precipitant to the other, the object (Boullata, 2005). If drug-nutrition interactions change therapeutic drug responses and/or compromise nutrition status irrespective of the precipitating factor or object they are considered clinically significant (Boullata and Hudson, 2012).

Drug metabolism occurs in two vital stages, the oxidation-reduction reactions involving the mixed function oxidase (MFO) system and the conjugation steps of the conjugase systems. The MFO occurs in the gut and is more susceptible to nutritional effects (Raiten *et al*., 2005; Wagner *et al*., 2001). As such, presystemic drug and other xenobiotic metabolism that occurs in the gut/or liver, is a vital predictor of drug transport and efficiency.

Occurring often than being thought are drug-food interactions. This is defined as any food, herbal medicine, or dietary supplements-induced changes in oral bioavailability

leading to changes in drug concentrations which affects efficacy and/or toxicity (Singh and Malhotra, 2004). Drug-food interactions have long been thought to be limited to the influence of the type or timing of meals on the absorption of a drug. In actual fact, food delays gastric emptying, raises pH of the proximal small intestine, increases hepatic blood flow and prolong the time of gastrointestinal transit, in comparison with fasting (Genser, 2008; Singh and Malhotra, 2004). Some foods affect the pharmacokinetics of orally taken drugs by acting either on the drug's intestinal metabolism or transport or both. Other foods or food components can cause reductions in plasma drug concentrations and pose a risk of treatment failure as a result of enzyme induction. The opposite of this can also occur as a result of enzyme inhibition by food or food products causing increased concentrations of drugs which may lead to life-threatening toxicities (Genser, 2008).

There are four key interactions that may take place among antiretroviral drugs, food and nutrition. These have been described by Castleman *et al.*, (2004);

- **Effects of food on drug metabolism**

The efficacy of certain ARVs can be enhanced or inhibited by some food and food products that affect the absorption, metabolism, distribution, or excretion of these ARVs. For instance a high energy, fat and protein meal decreases the absorption of indinavir (Pronsky *et al*., 2001) while a fatty meal enhances the bioavailability of tenofovir. The results of not properly managing these interactions is a reduction in the effectiveness of the therapy. Certain ARVs should therefore be taken on an empty stomach, others with food, and others with or without specific food types.

- **Effects of medication on nutrient absorption, metabolism, distribution, or excretion**.

Certain ARVs can affect the utilization of nutrients by altering how these nutrients are absorbed, metabolised, distributed or excreted. Some PIs for example ritonavir, indinavir and nelfinavir can cause alterations in lipid metabolism, leading to elevated levels of plasma triglycerides and cholesterol (Currier *et al*., 2003) thereby increasing the risk of coronary heart disease. The nutritional remedy for such an interaction will be to reduce the intake of saturated fats. Some PIs and NRTIs are found to cause lipodystrophy, a condition characterised by changes in body fat distribution (Currier *et al*., 2003). Certain protease inhibitors are known to cause alterations in the metabolism of carbohydrates, resulting in insulin resistance thereby increasing the risk of diabetes mellitus (Gelato, 2003).

- **Negative effects of medication side effects on food intake and nutrient absorption**

Reduced food intake and nutrient absorption can result from medication side effects and this aggravates nutritional problems such as wasting experienced by PLWHA. Loss of appetite, nausea and taste changes are some ARV side effects that may cause a reduction in food consumption while increase in nutrient losses can result in ARV-induced vomiting and diarrhoea. Zidovudine for instance can induce nausea, vomiting and loss of appetite while didanosine, another NRTI may cause side effects such as xerostomia, diarrhoea and anorexia (Pronsky *et al*., 2001). Appropriate dietary changes have to be made to in order to help manage some ARV side effects on PLWHA and to reduce the impact of these undesirable effects on their nutrition. Some ARVs can cause side effects that are not directly associated with food nonetheless, have nutrition

implications. It has been shown for instance that, some antiretroviral drugs raise the risk of osteopenia and osteoporosis among PLWHA. An appropriate nutritional response is to ensure adequate vitamin D and calcium intake by PLWHA with osteoporosis (Mondy and Tebas, 2003; Tebas *et al*., 2000). At one point during treatment, most people who take ARVs experience some side effects. The prevalence, frequency and severity of these side effects however differ among different individuals (Fellay *et al*., 2001; Pronsky *et al*., 2001; Carr and Cooper, 2000).

- **Production of unhealthy side effects as a result of the combination of certain foods and medication**

When combined with some foods, certain ARVs can produce dangerous side effects on PLWHA. For instance, consuming alcoholic drinks while taking didanosine can lead to pancreatitis which can be fatal (Pronsky *et al*., 2001). There is the need therefore for PLWHA to be given knowledge on the foods that are contraindicated with the drugs that they are taking in order to prevent further complications.

Apart from specific foods, certain individual nutrients can also influence the behaviour of drugs. The effect of an excess or deficiency of a particular macronutrient or micronutrient on drug metabolism may be contradictory (Raiten *et al*., 2005). The effect of vitamin A status on the cytochrome P450 activity regulation has been described by Ross and Zolfaghari (2004). Riboflavin, iron, niacin, copper and pantothenic acid, are essential as cofactors in many oxidation-reduction reactions of MFO system. Furthermore, proteins, lipids, zinc, calcium, magnesium, and Vitamins A, C, E and B6 are also vital for the preservation of membrane integrity and are critical supporting constituents of the MFO systems. The mechanism underpinning most drug micronutrient interactions is still unclear and controversial as some can cause a

reduction in the intestinal absorption of certain drugs while others may lead to nutrient depletion (Mouly *et al*., 2017; Karadima *et al*., 2016; Rogovik *et al*., 2010; Boullata, 2005; Mirtallo, 2004; Schmidt and Dalhoff, 2002). Studies have also assessed the relationship between macronutrients (such as fats and proteins) and the pharmacokinetics and bioavailability of ARV drugs. For instance, Aungst *et al*., (2002) described significantly higher circulating drug concentrations of NNRTI, efavirenz, in fed dog than that of fasted dogs. Another effect of macronutrients on the pharmacokinetics of drugs has to do with gastric emptying. Generally, fat delays gastric emptying to a greater extent than does protein or carbohydrate (Singh, 1999). An in-depth review article on the role of nutrition in drug therapy by Gura and Chan (2008) discusses most aspects of drug-nutrient interactions. Although obvious typical nutrient deficiency syndromes are not often seen, minor degrees of deficiencies are still presented as clinical signs. In some circumstances, these nutrient deficits are before now being classified as adverse drug effects. For instance, drug-induced osteomalacia can be caused by drug influence on vitamin D metabolism (Oscarson *et al*., 2006; Xu *et al*., 2006; Pascussi *et al*., 2005). Likewise, carnitine deficits may cause drug-induced hepatotoxicity and hyperammonaemia (Werner *et al*., 2007). Adverse drug effects associated with drug-induced nutrient deficits however remain to be studied (Boullata, 2013).

2.8.3 Recommended nutrients in HIV infection

A healthy diet for everyone, but more especially PLWHA, should include adequate quantities of both macronutrients (i.e. carbohydrates, proteins fats) and micronutrients (i.e. vitamins and minerals). At any time in their illness, PLWH are at risk of malnutrition. Most PLWHA who live in poverty experience poor nutrition as a result of

food insecurity which may be worsened by HIV infection (Gillespie and Kadiyala, 2005). As such, it will be very helpful to, as part of nutrition intervention for this group, inform them about their nutrient requirements and also advise them on increasing their caloric intake. The nutritional requirements of HIV-infected individuals are based on the stage of disease progression therefore required intake levels are recommended depending on the existence or non-existence of symptoms such as fever, diarrhoea, weight loss, and wasting.

2.8.3.1 Energy requirement in HIV positive individuals

Energy requirements are increased following HIV infection mostly through raising of resting energy expenditure and through complex metabolic changes that lead to weight loss and wasting mostly seen in AIDS. As such, asymptomatic HIV positive individuals need 10% extra energy than HIV-negative individuals of the same sex, age and physical activity level. A 20%-30% more energy is required by symptomatic HIV-positive individuals compared to HIV-negative individuals of the same sex, age and physical activity level (WHO, 2004). This is because, there is the need for more energy as the disease advances and opportunistic infections sets in.

2.8.3.2 Protein requirement among HIV positive individuals

Both asymptomatic and symptomatic HIV infected individuals do not require additional protein intake. Even though the onset of opportunistic infections causes loss of body nitrogen, there is insufficient information supporting the clinical benefits of increasing protein intakes. Recommendations by the WHO therefore is that, PLWHA should consume the same amount of protein that is sufficient to contribute the 12% to 15% of total energy intake as those people uninfected with HIV. A protein intake of 2-

2.5 g/kg/day should be a starting point for adults with HIV (WHO, 2005; FANTA, 2004; WHO, 2004; Keithley *et al*., 2000).

2.8.3.3 Fat requirement among HIV positive individuals

Recent WHO (2004) guidelines stated that there is no evidence that HIV infection calls for a change in fat requirements nonetheless, some ARV drugs or some symptoms of infections such as diarrhoea may necessitate alterations in the amount or timing of fat consumption in certain circumstances (FANTA, 2004).

2.8.2.4 Micronutrients requirement among HIV positive individuals

Micronutrients play an important part in the maintenance of immune function (including mucosal immunity) as well as general metabolism (Tang *et al*., 2005). Deficiencies in vitamins and minerals are common among PLWHA due to inadequate dietary intake, malabsorption, and infection (Garcia-Prats *et al*., 2010). Besides, chronic immune activation and increased oxidative stress in PLWH may lead to increased nutrient needs beyond the recommended intakes (Stephensen *et al*., 2006; Jahoor *et al*., 2003). Increased rate of HIV replication and accelerated cell death will result through oxidative stress. In spite of the above, the WHO recommendations do not support micronutrients intakes above the recommended levels of intakes for healthy HIV negative individuals of the same sex, age, and physical activity level (FANTA, 2004).

There are still inconsistencies in results of clinical trials for micronutrient supplementation and its benefits among PLHWA. Some studies (Fawzi *et al*., 2004; Jiamton *et al*., 2003; Shabert *et al*., 2001; Mocchegiani and Muzzioli, 2000; Müller *et al*., 2000) found benefits such as decreased viral load which improved CD4+ cell

numbers and resulted in less incidence of opportunistic infections hence, a slower disease progression rate. These studies also reported improved survival in adults with low CD4+ cell counts which prevented adverse birth outcomes during pregnancy and thus minimised mother-to-child HIV transmission among nutritionally weak women who have a more progressive HIV disease. In their review, Irlam *et al*., (2005) found no definite proof to show that micronutrient supplementation efficiently decreased morbidity and mortality among HIV positive adults but identified proof of advantages of vitamin A supplementation among children. They therefore suggested that, support should be given to the present WHO recommendations for the support and promotion of sufficient dietary consumption of micronutrients at RDA levels where such is possible. Micronutrient supplementation among PLWHA needs further investigation so as to assess its long term clinical advantages, negative effects and optimal formulation of micronutrient supplements. Most of the experiments carried out in this area used single micronutrients and small sample sizes therefore, information regarding combined outcome of multivitamin and multi-mineral supplementation on a large scale is still scanty.

Good nutrition can be best achieved through the consumption of various diets consisting of foods rich in micronutrients, especially iron, zinc, vitamins B6, B12, A, and selenium. Vitamins B and C and other water-soluble vitamins require daily incorporation in diet. Vitamin A and other fat-soluble vitamins can be taken every day on the least. By spending at least fifteen minutes in the sun every other day, vitamin D, which is important for bone development, can be obtained. Foods such as beans, leafy green vegetables, milk and milk products are good sources of calcium, which is essential in the development of bones. Vitamins A, B, C, and E are vital for the proper function of the immune system (Garcia-Prats *et al*., 2010).

Vitamin A is vital for vision and both vitamin A and C are essential for wound healing. The B vitamins play an important role in energy and erythrocyte production, as well as in growth. Vitamin E is essential as an antioxidant and in erythrocyte production. Zinc and selenium and other minerals such as copper, magnesium, potassium, phosphorus and iron are vital for the proper function of the immune system. These minerals are frequently exhausted during HIV infection. Intake of zinc and iron must be done with caution as they have both been linked with faster development from HIV infection to AIDS (Garcia-Prats *et al*., 2010).

It is advised that, micronutrient supplementation should be indicated only if an HIV-infected individual shows deficiency signs of a specific or multiple micronutrients, and must be done applying standard protocols (FANTA, 2004). Micronutrient supplementation is indicated in the United Kingdom (UK), in certain cases such as malabsorption, increased requirements and insufficient dietary intake (British Medical Association, 2008).

2.9 FREE RADICALS, REACTIVE SPECIES AND OXIDATIVE STRESS

Atoms and molecules which have unpaired electrons are generally very reactive and unstable. These are known as free radicals (Finkel and Holbrook, 2000). They are produced from molecules through the breaking of their chemical bonds such that, an electron is kept by each fragment. They can also be generated via the splitting of a radical to produce another radical; as well as through oxidation-reduction reactions (Halliwell and Gutteridge, 2007).

The terms that collectively describe free radicals and other non-radical reactive derivatives are reactive oxygen species (ROS) or reactive nitrogen species (RNS) (Agarwal *et al*., 2012). Reactive oxygen species are produced by living systems via

usual cellular metabolism (Birben *et al*., 2012). Reactive nitrogen species, including nitrogen based radicals and non-radicals for example nitric oxide, nitrogen dioxide radicals and peroxynitrite, are derived from nitric oxide and superoxide through inducible nitric oxide synthase (iNOS), and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase respectively (Mittler, 2002; McCord, 2000).

Other examples of free radicals are hydroxyl (OH•), superoxide ($O_2^{\bullet -}$), peroxyl (ROO•) and lipid peroxyl (LOO•). The following, lipid peroxide (LOOH), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), nitrous acid (HNO_2), dinitrogen trioxide (N_2O_3) and peroxynitrite ($ONOO^-$), are not free radicals but rather usually called oxidants because they can easily cause free radical reactions in living organisms (Genestra, 2007). Free radicals of biological origin are therefore very unstable molecules that have electrons ready to react with several organic substrates such as DNA, proteins, lipids and such that they can become stable, but in the process cause a cascade of chain reactions that result in cellular damage and disease.

Both enzymatic and non-enzymatic reactions generate ROS and RNS in the cell. The respiratory chain, phagocytosis, prostaglandin synthesis and cytochrome P450 system are examples of enzymatic reactions that produce free radicals (Halliwell, 2007; Pacher *et al*., 2007; Bahorun *et al*., 2006; Willcox *et al*., 2004). Free radicals can also be generated through the non-enzymatic reactions of organic compounds with oxygen and those initiated by ionizing radiations. Another non-enzymatic process of free radical generation is oxidative phosphorylation in the mitochondria (Genestra, 2007; Valko *et al*., 2007; Dröge, 2002).

Sources of ROS and RNS can either be endogenous or exogenous. Endogenous ROS/RNS are produced during infection, inflammation, aging, mental stress, immune

cell activation, ischemia, excessive exercise and cancer. Exogenous free radicals are formed when pollutants from water and air, alcohol, tobacco smoke, transition or heavy metals (Pb, Fe, Cd, As. Hg), some drugs (cyclosporine, bleomycin, gentamycin), cooking (smoked meat, used oil, fat,) industrial solvents and radiation, enter the body through various channels and are metabolized or decomposed (Genestra, 2007; Pacher *et al*., 2007; Valko *et al*., 2007; Young and Woodside, 2001).

Free radicals at low or moderate concentrations in the body are not harmful but rather are important for certain physiological activities. They serve in the maturation of cellular structures, cellular signalling systems, and induction of mitogenic response as well as acting as weapons for the defence system (Genestra, 2007; Pacher *et al*., 2007; Valko *et al*., 2007; Dröge, 2002; Young and Woodside, 2001).

Agarwal *et al*., (2012) defined oxidative stress (OS) as an imbalance between pro-oxidants and antioxidants which has been known to be involved in a wide range of processes, diseases and syndromes including mutagenesis, cell transformation and cancer in recent years (Poljsak and Milisav, 2012). It can arise when cells are unable to effectively contain the excess free radicals produced or when damage caused by ROS/RNS cannot be repaired. Through lipid peroxidation, excess hydroxyl radicals and peroxynitrites for instance can cause damage to lipoproteins and plasma membranes. This interrupts the membrane lipid bilayer arrangement that may deactivate membrane bound receptors and enzymes which increases tissue permeability. Lipid peroxidation produces malondialdehyde (MDA) alongside conjugated diene compounds, which are mutagenic and cytotoxic. The reaction occurs as a radical chain reaction and affects a large number of lipids.

Structural changes and loss of enzyme activity occur when proteins are damaged by ROS/RNS (Halliwell, 2007). Oxidative damage to DNA occurs in various ways such as

damage to bases, single or double stranded DNA breaks, purine, pyrimidine or sugar bound modifications, deletions or translocations, mutations, and cross-linking of proteins. Most of these DNA alterations are highly significant to aging, carcinogenesis, cardiovascular, neurodegenerative and autoimmune diseases.

The body DNA repair enzymes and/or antioxidants are critical to neutralizing these attacks (Genestra, 2007; Halliwell, 2007; Pacher *et al*., 2007; Willcox *et al*., 2004). Various chronic and degenerative diseases as well as aging process and some acute pathologies such as trauma and stroke, can be prompted by oxidative stress if not properly regulated.

2.9.1 Reactive species generation and oxidative stress in HIV infection

So far, several lines of evidence demonstrate that HIV infection activates marked oxidative stress in both laboratory models and in *in vivo* infection models. Higher ROS production in monocytes is exhibited by people infected with HIV (Elbim *et al*., 1999) and severely raised levels of oxidized nucleic bases such as 8-oxoguanine (8-oxoG) and lipid peroxidation products, including MDA in plasma, and alkanes in the breath (Watanabe *et al*., 2016; Kalinowska *et al*., 2013; Teto *et al*., 2013; Awodele *et al*., 2011; Wanchu *et al*., 2009; Aukrust *et al*., 2005; Gil *et al*., 2003).

Oxidative stress is induced by HIV-1 through the deregulation of oxidative stress pathways leading to an increase in ROS production and by inducing mitochondrial dysfunction (Deshmane *et al*., 2009). Generation of ROS in several cell lines of lymphoid origin is enhanced by the envelope protein Gp120 (Banerje *et al*., 2010). The viral Tat protein also induces a heightened ROS production in HIV-infected patients. This is established by mitochondrial manufacture of superoxide anion (Sacktor *et al*., 2004) after which nuclear factor κappa B (NF-κB) is activated (Schreck *et al*., 1991)

thereby increasing HIV transcription. Activated polymorphonuclear leukocytes and HIV-activated macrophages through tumour necrosis factor (TNF-α) release, also contributes to the production and build-up of ROS (Robinson, 2009).

Apoptosis of CD4+ T cells, the pathogenesis of loss of immunity in chronic HIV infection is influenced by ROS production and oxidative stress (Okoye and Picker, 2013). The resultant effect of ROS production on the organism is a deficiency in its antioxidant capability, partly due to extreme use of antioxidant molecules in an attempt to guard the cells against ROS-induced injury (Suresh *et al*., 2009). This further contributes and enhances the pro-oxidative status.

Many *in vitro* studies have associated oxidative stress to numerous features of HIV pathogenesis, including stimulation of HIV replication, quantitative and functional damage of CD4+T cells, perturbed immune response, and toxicity of antiretroviral drugs (Gendron *et al*., 2011; Manda *et al*., 2011; Aukrust *et al*., 2005; Hulgan *et al*., 2003). Oxidative stress has also been revealed to be involved in some HIV-associated diseases, such as HIV dementia (Steiner *et al*., 2006).

It has been revealed that PLWH and AIDS patients have increased oxidative stress biomarkers compared to HIV negative controls (Suresh *et al*., 2009). In their study, Masiá *et al*., (2016) concluded that, oxidative stress is a predictor of mortality in HIV-infected patients.

Malnutrition has the tendency to cause oxidative stress. The lack of certain minerals and vitamins in the diet will lead to their deficiency and adversely influence redox status of the body. For instance, selenium deficiency will cause a reduction in the production of selenoproteins and glutathione which may adversely influence oxidative balance and result in oxidative injury on the cell membrane (Sharma, 2014).

Furthermore, oxidative stress induced by zidovudine (AZT/ZDV) has been reported to cause inhibition of mitosis and protein synthesis. This effect is usually exerted on B cells, leading to a reduction in their number and hence their capacity to produce enough antibodies leading to immunodeficiency (Sharma, 2014). More evidence is required to fully comprehend the means by which these play off despite the current theoretical etiopathogenic role of oxidative balance in HIV infection.

2.9.2 Reactive species generation and antiretroviral drugs

Among key discoveries in the redox biology of the HIV-1 virus is that of the knowledge about the generation of oxidative stress during antiretroviral therapy (Ivanov *et al*., 2016). So far, several accounts about this have shown that, NRTIs, NNRTIs and PIs, initiate huge ROS production in different cell types (Weiß *et al*., 2016; Nagiah *et al*., 2015; Wang *et al*., 2013; Manda *et al*., 2011; Chandra *et al*., 2009; Hurwitz *et al*., 2004; Mondal *et al*., 2004). Most of these studies (Adikwu *et al*., 2013; Mandas *et al*., 2009; Masiá *et al*., 2007; Ngondi *et al*., 2006) have reported an upsurge in oxidative stress additional to the persistent redox imbalance associated with HIV-1 infection established by a rise in oxidants and a reduction in serum antioxidant levels. From their study, Aukrust *et al*. (2003) suggested that, even though HAART has been successful, it was also accompanied with an enhanced oxidative stress and a disturbed glutathione metabolism. Experiments in the ART-exposed cell lines and laboratory animals revealed that the enhanced production of the oxidized metabolites come about through the mitochondrial interference (Mandas *et al*., 2009).

Mitochondrial dysfunction due to ART, according to Day and Lewis (2004), results from the changed replication of mitochondrial DNA and inhibited oxidative

phosphorylation. The duration of antiretroviral therapy is correlated with some of the above mentioned dysfunctions (van Vonderen *et al*., 2009; Sevastianova *et al*., 2005).

Tabe *et al*., (2015) specifically explained how this oxidative imbalance happens. They reported that since ARVs were not made to distinguish their targets, nuclear DNA from the mitochondrial DNA, it is likely that mitochondrial DNA is attacked during ARV activities. The mitochondrial DNA polymerase gamma (DNA pol-γ) is the enzyme that replicates and maintains mitochondrial DNA (Valle *et al*., 2013). The proteins encoded by mitochondrial DNA partake in electron transport complexes of oxidative phosphorylation. They thus, inhibit DNA pol-γ, causing mitochondrial DNA reduction and a change in oxidative phosphorylation and consequent energy deprivation. All of these contribute to accumulation of free radicals (Valle *et al*., 2013) due to the obstruction of important reactions of the electron transport chain (Jiang *et al*., 2010) particularly those of complexes I and III.

It is generally accepted that oxidative species produced from the components of ART and the long term use of ARV may contribute to the development of cardiovascular diseases, central nervous system pathologies, cerebrovascular diseases and renal disorders. Some of the antiretroviral drugs, such as 2', 3' dideoxycytidine (ddC), can penetrate the blood brain barrier (BBB) and trigger oxidative stress also in the brain (Kyselova, 2011; Opii *et al*., 2007; Rodriguez *et al*., 2006). The precise result of oxidative stress on the efficacy of ART and HIV-1/AIDS progression and the molecular mechanisms of the redox imbalance in ART-treated HIV positive people still remains unclear therefore requiring more comprehensive research (Ivanov *et al*., 2016).

2.10 ANTIOXIDANTS

The body is equipped with an antioxidant system to counter oxidative stress. These antioxidants can either be acquired externally from foods (exogenous antioxidants), or produced naturally within the body (endogenous antioxidants). Antioxidants function in delaying, inhibiting and neutralizing the deleterious actions of excess free radicals thereby protecting cells from lethal free radicals effects and contributing towards disease prevention.

2.10.1 Classification of antioxidants

Endogenous antioxidants are classified as either enzymatic or non-enzymatic antioxidants. The enzymatic defence is made of an enzyme system that is directly involved in the neutralization of ROS and RNS. They include superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GRx) and catalase (CAT) (Genestra, 2007; Halliwell, 2007; Pacher *et al*., 2007; Bahorun *et al*., 2006; Valko *et al*., 2006; Valko *et al*., 2005; Willcox *et al*., 2004).

The primary source of defence against free radicals is SOD which catalyses the decomposition of superoxide anion radical ($O^{2\bullet-}$) into hydrogen peroxide (H_2O_2) by reduction. The oxidant formed (H_2O_2) is converted into water (H_2O) and oxygen (O_2) by catalase (CAT) or glutathione peroxidase (GPx). The selenoprotein enzyme GPx, eliminates H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, uses NADPH as a source of reducing power to regenerate GSH from GSSG. Furthermore, while oxidizing glutathione (GSH), GPx also reduces lipid or non-lipid hydroperoxides (Genestra, 2007; Halliwell, 2007; Pacher *et al*., 2007; Bahorun *et al*., 2006; Willcox *et al*., 2004 Dröge, 2002).

Conversely, non-enzymatic antioxidants can also be grouped into metabolic and nutrient antioxidants. Metabolic antioxidants are made via metabolic activities in the body and thus are endogenous antioxidants. Examples include glutathione, L-ariginine, lipoid acid, melatonin, metal-chelating proteins, uric acid, bilirubin, transferrin and coenzyme Q_{10} (Willcox *et al*., 2004; Dröge, 2002). Nutrient antioxidants on the other hand are exogenous antioxidants and are compounds which cannot be made in the body which must be supplied via foods or supplements. Examples are vitamin C, vitamin E, omega-3 and omega-6 fatty acids, carotenoids, trace metals (zinc, manganese, selenium) and flavonoids.

2.10.2 Mode of action of antioxidants

An antioxidant becomes oxidized itself after neutralising a free radical therefore resources need to be restored in the body constantly. In this regard, an antioxidant can be effective against free radicals in a particular system and ineffective in another system. An antioxidant can generate ROS/RNS in some circumstances, thereby acting as a pro-oxidant in that system (Young and Woodside, 2001).

There are two ways in which the antioxidant system can function. These are chain-breaking or prevention.

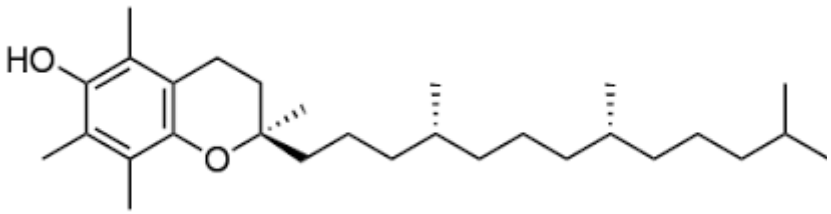
- **Chain-breaking:** A new radical is produced when a radical picks or releases an electron. This newly created radical exerts similar action on another molecule and this goes on until the free radical created is either stabilized by a chain-breaking antioxidant such as vitamins C, E, or carotenoids, or it just breaks down into a harmless byproduct. A classic example of such a chain reaction is lipid peroxidation.

- **Prevention:** In the preventive system of antioxidant function, antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase can prevent oxidation by slowing the rate of chain initiation, either by scavenging free radicals or by stabilizing transition metal radicals such as iron and copper (Young and Woodside, 2001).

2.10.3 Specific antioxidant micronutrients considered in this study

2.10.3.1 Vitamin E

Vitamin E is a fat-soluble vitamin, which primarily functions as an antioxidant. It is thus unique amongst other vitamins that mostly act as cofactors or have specific metabolic functions (Traber and Atkinson, 2007). Vitamin E is a chiral compound that has eight stereoisomers: α, β, γ, δ tocotrienol and α, β, γ, δ tocopherol, with each having on the chromanol ring a different number of methyl groups i.e. trimethyl (α−), dimethyl (β− or γ−), and monomethyl (δ−) (Traber, 2012). Among them, the most preferred form of vitamin E in the human body is α-tocopherol (Bendich, 2001; Institute of Medicine, 2000). Research in both humans and animals has shown that natural dextrorotatory d-α-tocopherol is almost twice as effective as synthetic racemic dl-α-tocopherol (Nguyen *et al*., 2006).



Source: *National Center for Biotechnology Information PubChem Compound Database*

Figure 2.2 Chemical structure of vitamin E (α –Tocopherol)

All vitamin E forms are synthesised by plants that serve as main food sources such as, nuts, wheat germ oil, vegetable oils, whole grains, fruits, cereals, meat, eggs and poultry (Willcox *et al*., 2004). Esters of vitamin E such as tocopheryl acetate are more

stable, even in oxidizing conditions than the free forms of the vitamin such as tocopherols that are fairly stable but can be damaged by oxidation. Vitamin E can be destroyed through deep-fat frying but cannot be lost through boiling in water because it is insoluble in water.

The most important lipid-soluble antioxidant in the cell is vitamin E, functioning as a potent peroxyl radical scavenger. From its location in the lipid portions of the cell membrane, it protects unsaturated phospholipids of the membrane from oxidative degeneration due to ROS and other free radicals through a chain-breaking antioxidant action. It also exerts this antioxidant action on plasma lipoproteins (Traber and Atkinson, 2007).

Peroxyl radicals (ROO•) following their generation react thousand times faster with vitamin E (Vit E-OH) compared to polyunsaturated fatty acids (PUFA or RH); hence, vitamin E protects lipoproteins and membranes from the chain reaction of lipid peroxidation. Hydroperoxide (ROOH) and the tocopheroxyl radical (Vit E - O) are made as the peroxyl radical reacts with the hydroxyl group of tocopherol. The tocopheroxyl radical reacts with a hydrogen donor, (AH) such as vitamin C, where it oxidizes the latter and returns vitamin E to its reduced state. It should be noted that, lipid peroxidation can be reinitiated by free metals, such as copper or iron via reaction with ROOH to form an alkoxyl radical. Moreover, Vit E - O• can reinitiate lipid peroxidation if other antioxidants are unavailable (Thomas and Stocker, 2000).

Vitamin E metabolism is limited and is mediated by cytochrome P450s (CYP). It is first oxidized into the biologically inactive tocopheryl quinone, which is then reduced to tocopheryl hydroquinone. The main route of removal from the body is through faeces in the form of glucuronic acid conjugate of the hydroquinone. Very small portion of vitamin E side-chain metabolites (tocopheronic acid and tocopheronolacton),

considering normal intakes are excreted in the urine as water-soluble (Sontag and Parker, 2002; Brigelius-Flohe and Traber, 1999).

Vitamin E is among the less toxic vitamins with an upper limit of 1000mg/day for adults. Relatively high intakes of at least 100 times the nutritional requirements does not seem to harm both humans and animals. Notwithstanding this, very high doses of vitamin E can reduce the body's ability to utilise other fat-soluble vitamins. Impaired bone mineralisation, hepatic vitamin A storage, and blood coagulation was seen in animal fed with excessively high amounts of vitamin E (Traber, 2007). Inconsistent data concerning high-dose supplementation of vitamin E and increased mortality of patients with inflammatory joint diseases, cardiovascular disease, and cancer have been seen in the recent past years. Further studies into a questionable casual relationship of vitamin E supplementation and increased mortality is required (Gerss and Kopcke 2009).

Ghana has adopted the Codex Alimentarius Commission's Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) nutrient reference value (NRV) recommendation of 9mg/day (Codex Alimentarius Commission, 2016).

The clinical indicators of vitamin E deficiency vary substantially. In general, certain organ systems such as vascular, the neuromuscular and reproductive systems are the targets of deficiency which may take 5 to 10 years to develop. Main symptoms include loss of deep tendon reflexes, impaired vibratory and position sensation, changes in balance and coordination, muscle weakness, and visual disturbances (Sokol, 2001). These have taken place in people with lipid malabsorption due to biliary atresia or exocrine pancreatic insufficiency or with lipid transport disorders like abetalipoproteinemia.

A deficiency of vitamin E at the cellular level is accompanied by an increase in lipid peroxidation of the cell membrane. This makes the vitamin E-deficient cells open to oxidative stress, leading to rapid injury and necrosis. Plasma vitamin E is however depleted rapidly by oxidative stress in humans (Bruno *et al*., 2006; Bruno *et al*., 2005) and adequate vitamin consumption prevents faster vitamin E depletion (Bruno *et al*., 2006).

2.10.3.2 Zinc

Zinc is the second most abundant trace mineral in the human body secondary only to iron (Mahan and Raymond, 2016). It is found both in animal and plant food sources such as red meat, fish, poultry, liver, various shellfish types (such as oysters), soy products, whole grain cereals, dry beans, and nuts, zinc fortified cereals and milk and milk products (Mahan and Raymond, 2016). Since most of the food sources are protein based, zinc intake mostly correlates with protein intake.

A dietary reference intake (DRI) of 11mg/day is estimated for adolescent and adult males. A lower DRI of 8 to 9mg/day is estimated for adolescent and adult females due to lower body weight compared to males. For pre-adolescents, DRI for zinc is estimated to be 8mg/day. Infants have a DRI of 2mg/day and 3mg/day for the initial and second six months of life respectively (Mahan and Raymond, 2016).

A small amount of zinc absorption in monogastric animals occurs in the stomach, with the duodenum, jejunum, and ileum, being the main absorption sites in the gut (Holt *et al*., 2012). The amount of zinc that is available from the matrix of a food substance and the total zinc content of the food is a primary determinant of the bioavailability of zinc (Hambidge *et al*., 2010; King, 2010). The presence or absence of interfering or enhancing substances is another determinant of zinc bioavailability and absorption. For

instance, phytate (myoinositol hexaphosphate), a plant based compound found in roots, tubers and seeds, significantly competes with zinc at absorption sites and in the process prevents zinc absorption (Hambidge *et al*., 2010; Lönnerdal, 2000). On the other hand, certain food substances such as liver, meats, egg, and seafood are known to be excellent zinc sources as they relatively do not contain chemicals that will inhibit zinc absorption and also because they contain special amino acids that enhance zinc solubility (Krebs, 2000).

Majority of physiological processes require zinc, as such, its metabolic and regulatory roles are areas of substantial study. Nearly 10% of mammalian encoded proteins require zinc for their appropriate structure and function (Andreini *et al*., 2006). The functions of zinc as a cofactor for over 2000 transcription factors and 300 enzymes as well as an essential mediator in cellular signalling, makes it a vital mineral for human health (Roshanravan *et al*., 2015; Jurowski *et al*., 2014). Zinc has multiple effects on the endocrine system. These include a role in the metabolism of androgen hormones, oestrogen, and progesterone, as well as with the prostaglandins. Moreover, zinc plays critical roles in the secretion of insulin, and regulation of thymic hormones (Pfaender *et al*., 2017).

It functions principally to boost insulin action. Zinc provides structural stability to cell membranes serving as an anti-inflammatory agent and also a vital regulator of gene expression (Fung *et al*., 2015; Foster *et al* 2014; Jansen *et al*., 2012). Zinc also functions in the immune system since immunological events such as haematopoiesis, humoral immunity cell function and survival and cytokine secretion of people are affected by their zinc status (Foster and Samman, 2012; Fraker and King, 2004).

Zinc functions in protecting cells against oxidative stress even though it is not in itself, an antioxidant as it does not partake in oxidative-reduction reactions. It is thus

considered a pro-antioxidant since it protects cells against detrimental effects of oxygen radicals produced through immune activation (Maret, 2006; Bray and Bettger, 1990). This antioxidant role has been widely studied and it occurs through various mechanisms.

Similarly, zinc serves as a co-factor to essential enzymes that contribute to the appropriate operation of the antioxidant defence system. It further guards cells against oxidative injury since it acts in the stabilization of membranes, inhibits the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH-Oxidase), a pro-oxidant enzyme, and prompts the synthesis of metallothionein, a cysteine-rich low molecular weight protein. Metallothionein however, functions in the decrease of hydroxyl radicals (OH) and in the sequestration of the ROS created during stress situations (Ruz *et al*., 2013; Chasapis *et al*., 2012).

Zinc forms an important structural component of superoxide dismustase, an enzyme found in a cell's cytoplasm. The enzyme possesses an active centre with zinc and copper ions and supports the transformation of two superoxide radicals to hydrogen peroxide and a molecular oxygen thereby decreasing the toxicity of ROS and converting a highly reactive species to a less injurious one (Cruz and Soares, 2011).

Zinc also acts as antioxidant by affecting the expression of the rate limiting enzyme of glutathione *de novo* synthesis, glutamate-cysteine ligase. This produces a double result of zinc in neutralizing free radicals directly by glutathione or indirectly as a glutathione peroxidase cofactor (Eide, 2011). Zinc also controls the total glutathione concentration in cells (Foster and Samman, 2010).

The most abundant, nonenzymatic zinc containing protein is metallothionein. Under normal physiological conditions, zinc is bound to this protein. When cells suffer

oxidative stress, zinc is released from the metallothionein complex and is redistributed in the cells to exert antioxidant effects (Özcelik *et al*., 2012; Maret and Krężel, 2007).

Even though zinc is important for appropriate immune operative and for the integrity of mucosal surfaces, high zinc consumption was shown to have possible deleterious effects on HIV progression in early observational studies (Tang, 1996; Tang *et al*., 1993). Zinc fingers are formed when zinc binds to the viral nucleocapsid protein 7 (Ncp7). These zinc fingers are important in pro-viral DNA and the action of reverse transcriptase. There is therefore the fear that, zinc supplementation might encourage viral replication instead of inhibiting it (Tanchou *et al*., 1998; Bess *et al*., 1992).

Zinc supplementation was shown to reduce markers of oxidative stress and zinc deficiency results in an increase in oxidative stress (Sun *et al*., 2015). In order to ensure the appropriate functioning of the antioxidant defence system, it is important to maintain sufficient concentrations of zinc in the cell compartments.

Zinc deficiency is associated with numerous clinical signs. For instance, slight zinc deficiency may result in impairment of memory, depressed immunity, neurosensory problems such as impaired smell and taste as well as onset of night blindness, and reduced spermatogenesis in males (Shankar and Prasad, 1998; Walsh *et al*., 1994). Severe zinc deficiency is associated with a more severely depressed immune function as a result of frequent infections, dermatitis, alopecia, diarrhoea, and mental disturbances (Shankar and Prasad, 1998).

While dietary factors usually cause zinc deficiency, several inherited defects of zinc deficiency have been identified. Notable among them is Acrodermatitis enteropathica (AE). In humans, it is the most common presentation of inherited zinc deficiency (Küry *et al*., 2015). The clinical characteristic of AE is impaired intestinal absorption of zinc, resulting in a triad of symptoms: alopecia, dermatitis, and gastrointestinal (GI)

problems such as intractable diarrhoea. Other symptoms such as irritability, mental depression, loss of appetite, behavioural problems, and reduced immune function may frequently occur.

There have been reported cases of acute zinc toxicity in humans due to exposure to extreme quantities of zinc even though this is rare. This exposure can occur through inhalation, through the skin, and by ingestion. The upper limit (UL) for zinc in adults is 40 mg/day and oral ingestion of toxic quantities of zinc i.e. 100 to 300 mg/day, is uncommon. Metal fume fever as a result inhalation of zinc-containing smoke, which normally contains zinc oxide is considered the most common form of zinc toxicosis. Signs of this disease include profuse sweating, hyperpnoea, and general weakness and can develop within 8 hours of exposure. These have been reported to resolve within 1-4 days following the removal of the person from the zinc-contaminated environment. Zinc exposure through the skin however is not seen to represent an important toxicological risk (Plum *et al*., 2010).

2.11 HIV INFECTION AND ANTIRETROVIRAL THERAPY AND EFFECTS ON LIVER FUNCTION

At present, one of the main causes of morbidity and mortality in HIV-infected individuals are hepatic events (Palella *et al*., 2006; Weber *et al*., 2006). Both HIV infection and antiretroviral drugs have the tendency to cause liver damage and consequent effect on liver function. Liver injury according to clinical terms, is said to occur when there are abnormalities in liver tests (Núnez, 2006) and its severity ranges from asymptomatic to liver decompensation; and the outcome, from spontaneous resolution to liver failure and death (Clark *et al*., 2002).

Antiretroviral drug related liver injury (ARLI), which is a type of drug-related liver injury is defined using the rise of liver enzymes in serum, with alanine aminotransferase (ALT) characteristically greater than aspartate aminotransferase (AST). The criteria used in clinical studies to categorize the severity of hepatotoxicity has seen a wide variability to date (Soriano *et al*., 2008). Certain studies have adopted an absolute threshold (e.g. >100IU/ml), regardless of baseline liver function tests. Others have also used ALT levels that are two times the upper limits of the normal (ULN) to determine the severity of hepatotoxicity.

The most accepted criteria however is that of the AIDS Clinical Trial Group scale of liver toxicity. By this criteria, hepatotoxicity is said to have developed when patients who had transaminases within normal limits at baseline experience a rise in ALT and/or AST higher than the ULN. They defined severe hepatic injury as grade 3 or 4 change in AST and/or ALT levels during antiretroviral treatment. In the case where AST and ALT grades were conflicting, the highest was used for the classification (Soriano *et al*., 2008; Núnez, 2006).

Abnormalities in liver function test results should however be interpreted carefully. Several drugs (e.g. nevirapine and less often efavirenz) cause a rise in serum γ-glutamyltranspeptidase (GGT) levels. This is frequently mistaken as an indicator of liver damage, but the isolated rise of this enzyme really reflects enzyme induction. Elevated bilirubin levels should not be used in isolation to predict liver injury since hyperbilirubinaemia can occur due to haemolysis, fasting and drug such as indinavir and atazanavir (Lankisch *et al*., 2006; Zucker *et al*., 2001). Other factors can predispose one to ARLI. For instance, the use of alcohol, a known hepatotoxin, has been linked with an increased risk of ARLI in the studies that have researched this subject (Núñez *et al*., 2001). Prolonged utilization may also predispose to hepatocyte injury by

increasing oxidative damage to mitochondrial DNA and exhausting glutathione, a vital scavenger of free oxygen radicals' stores. The use of cocaine and ecstasy may also lead to acute hepatitis. Hepatotoxicity caused by cocaine may occur through a toxic oxidative metabolite, which induces metabolic damage (Campos *et al*., 2002).

Drug induced liver injury (DILI) can be considered as predictable or unpredictable with associated high or low incidences of occurrence respectively. Predictable hepatotoxic reactions are dependent on the dosage of drug and are independent of host factors, with a typical example being acetaminophen (paracetamol) toxicity (Kaplowitz, 2001). Four known mechanisms are involved in the development of ARLI. These are, mitochondrial toxicity, immune reconstitution, metabolic host–mediated and hypersensitivity (Soriano *et al*., 2008).

Drug-induced liver injury as a result of the beginning of ART is more frequent in HCV/HIV co-infected patients than in those with just HIV infection. HCV/HIV co-infected individuals with advanced liver disease (e.g. end-stage liver disease, cirrhosis) are at the highest risk for DILI (Aranzabal *et al*., 2005). A decrease in the likelihood of ARV-associated DILI however, may be achieved when HCV infection is eradicated through treatment (Labarga *et al*., 2007).

2.12 OXIDATIVE STRESS AND ORGAN DYSFUNCTION

A major organ attacked by ROS is the liver (Sánchez-Valle *et al*., 2012) with its parenchymal cells as the primary cells exposed to the liver injury. Production of ROS by the mitochondrion, peroxisomes and microsomes of parenchymal cells is mostly linked to the expression of liver fatty acid oxidation gene. Additionally, other hepatic cells such as the Kupffer cells, endothelial cells, and hepatic stellate cells are also potentially exposed and sensitive to oxidative stress inducing molecules. When

oxidative stress induces Kupffer cells, different types of cytokine such as TNF can be generated and this potentially may cause inflammation and apoptosis. Lipid peroxidation due to oxidative stress initiates the propagation and collagen synthesis of hepatic stellate cells (Cichoż-Lach and Michalak, 2014; Sakaguchi *et al*., 2011; Cederbaum *et al*., 2009). Homeostatic balance is disrupted in the event of excessive ROS leading to oxidative stress, a stage that plays a crucial role in liver diseases and other chronic and degenerative disorders (Li *et al*., 2014). Drug toxicity also targets the liver most frequently than other organs. Radical species generation may be an indicator of the hepatotoxic potential and hepatotoxicity of drugs (Videla, 2009).

Oxidative stress further significantly modulates pathways that control normal biological functions apart from triggering hepatic injury. By causing irreparable change to proteins, lipids and DNA, oxidative stress is regarded as one of the pathological mechanisms that leads to the start and development of different liver diseases, such as chronic viral hepatitis, alcoholic liver diseases and non-alcoholic steatohepatitis since these pathways control genes transcription, protein expression, cell apoptosis, and hepatic stellate cell activation (Feng *et al*., 2011; Singal *et al*., 2011). There have also been suggestions of the existence of complicated interrelations between pathological factors, free radicals, inflammation and immune responses (Singal *et al*., 2011). Furthermore, damage to extra-hepatic organs, such as the kidneys and brain can occur due to the systemic oxidative stress that arises during liver disease (Palma *et al*., 2014).

CHAPTER THREE

SUBJECTS AND METHODS

3.1 STUDY DESIGN

This is a hospital-based, cross-sectional study. This study design was applied due to limited time for the study.

3.2 STUDY SITE

The Volta Region has a total landmass of about 20,570 square kilometres, which is 8.7% of the total landmass of Ghana. It is located between latitudes 5º 45"N and 8º 45" N along the southern half of the eastern border of Ghana, where it shares borders with the Republic of Togo. The region also shares borders with Greater Accra, Eastern and Brong Ahafo Regions to the east, and to the north, it shares borders with the Northern Region. The region has the Gulf of Guinea at the southern part. The climate here is tropical and is characterized by moderate temperatures ranging from 21˚C through 32˚C for most of the year. Two rainfall seasons exist in the region with the first starting from March to July and the second from mid-August to October. The 2010 census report puts the regional population at 2,118,252 people with a growth rate of 2.5% (Ghana Statistical Service, 2010). The region is divided into 25 Municipal and District assemblies headed by Chief Executives. Ho is the capital city of the region where the Volta Regional Hospital is located.

This study was carried out at the ART clinic of the Volta Regional Hospital, Ho, Ghana. The Volta Regional Hospital (VRH) is a government owned regional referral hospital that takes referrals from health centres, clinics and district hospitals in the Volta region. The hospital currently has a bed capacity of 240. Specialist services provided by the hospital include obstetrics and gynaecology, internal medicine,

paediatrics, dental care, ear, nose and throat, accident and emergency, radiology and imaging and physiotherapy. There is an antiretroviral therapy (ART) unit which runs the ART and TB clinics. The ART unit of the VRH was started in 2006 initially to manage HIV and AIDS patients.

3.3 STUDY POPULATION

The study population was made up of both adult males and females who have been diagnosed of HIV and are attendants of the Volta Regional Hospital ART clinic. Their age ranged from 24 through 88 years. All the participants have been on antiretroviral drugs (ARVs) for at least 3 months.

3.4 SAMPLING TECHNIQUE

The Volta Regional Hospital was chosen because it is the regional and referral hospital of the Volta Region. A list of possible participants who met the inclusion criteria set for the study, was provided by officials of the Antiretroviral Unit. The population for the study was randomly sampled from this list by selecting every second name after the previous.

3.5 SAMPLE SIZE DETERMINATION

Cochrane's formula was applied to determine the sample size as follow:

$$Sample\ size = \frac{Z_{\alpha/2}{}^2\, p(1-p)}{d^2}$$

$Z_{a/2} = Z_{0.05/2} = Z_{0.025}$ = confidence interval of 95% = 1.96 (From Z scores table) at type 1 error of 5%

p = Estimated prevalence of study population = 1.47% = 0.015

d = marginal error = 0.05

Therefore formula became;

$$Sample\ size = \frac{(1.96)^2 \times 0.015(1-0.015)}{0.05^2} = 22.7$$

The minimum number of participants required for the study is 23, however this number was increased to 103.

3.6 RECRUITMENT OF STUDY PARTICIPANTS

The study objective and procedures were fully explained to study participants prior to their recruitment and their informed consent obtained. They were informed about the content of the interview to enable them understand the procedures and to ensure their full approval. The importance of the study as well as possible risks that may be involved were also made known to the participants. Participation was totally voluntary, thus, individuals had the right to or not to take part in the study and could opt out at any time of the study if they so wished. Only consenting individuals were interviewed, measurements taken and samples collected. The data was handled by the researcher alone, which ensured and guaranteed confidentiality.

A special coding system of identification was adopted that masked study participants' names and samples thereby protecting their identities. Findings from the data collected were communicated to study participants through their physician.

3.6.1 Inclusion criteria

- Individuals who were18 years old and above and had been diagnosed with HIV for at least six (6) months prior to the beginning of data collection and were attendants at the Antiretroviral Clinic of the hospital.
- Individuals who have been on antiretroviral therapy for at least three (3) months.

- Individuals who gave their consent to participate in the study.

3.6.2 Exclusion criteria

- Individuals who were smoking and/or beginning an antioxidant vitamin therapy prior to the study.
- Individuals who have the following conditions: Hyperlipidaemia, liver dysfunction and intractable diarrhoea (at least six liquid stools daily).

3.7 DATA COLLECTION AND TOOLS

Data collection spanned from 2nd February, 2017 to 30th March, 2017. The categories of information obtained include, socio-demographic data, anthropometric data and biochemical data. Others are dietary intake information and medical history. Pretested structured questionnaires were used to collect the socio-demographic and medical information. Secondary data that provided information on participant's past and current medical histories and drug history were obtained from study participant's medical records from the ART unit of the hospital.

3.7.1 Pretesting of questionnaires and survey tools

Pretesting of questionnaire and survey tools was done for one day using ten HIV positive individuals. This led to the rephrasing of certain questions to make them clearer and also get conversant with the use of some of the instruments for the study.

3.7.2 Socio-demographic information

Socio-demographic information such as age, level of education, occupation, income status, marital status and location were collected.

3.7.3 Anthropometric assessment

3.7.3.1 Weight

The weight of study participants was measured with a well calibrated digital Seca® scale by Seca, UK. All measurements were taken to the nearest 0.1 kilogram (kg). The subject was asked to remove all excess clothing, and other materials from pockets. Participants were made to stand upright on the scale bare footed. The weight of the subject was read and recorded in kilograms. The process was repeated once and the average weight in kilogram was determined.

3.7.3.2 Height

Height was measured using a Seca® stadiometer by Seca, UK. The stadiometer was placed against a wall and on a flat floor. The participants were asked to take off their footwear and hang their arms loosely. Their heads, scapulae, heels and buttocks were made to touch the vertical board of the stadiometer. Their heads were made erect with their eyes focused straight ahead and they were asked to inhale. The rod was lowered to the most superior point of theirs head, compressing their hair. The readings were taken twice and recorded in centimetre (cm) and average calculated and recorded to the nearest 0.1m.

3.7.3.3 Body mass index (BMI)

Body mass index was calculated using the formula, weight divided by the square of the height. The WHO classification of BMI was used to classify participants into various weight statuses respectively.

Table 3.1 WHO classification of BMI

BMI (kg/m^2)	WHO CLASSIFICATION
Less than 18.5	Underweight
18.5 to 24.9	Normal weight
25 to 29.9	Overweight
Above 30	Obese

Source: WHO, 1998

3.7.4 Dietary assessment

A food frequency questionnaire (FFQ) containing list of common foods that are rich sources of antioxidant nutrients was used to assess the frequency of intakes of study participants. The FFQ contained frequencies in categories ranging from 'daily' to 'never'. A 3-day, 24-hour dietary recall based on two weekdays and a weekend was used to assess actual dietary intakes for antioxidant micronutrient. During the interview, respondents were asked to recall food and beverage intake of the previous 24 hours for two week days and one weekend day. Household handy measures shown in plate 3.1 were used to estimate the quantities of the various foods consumed. All dietary assessments were conducted by the principal investigator with assistance two dieticians. A conversion table, the *Ghana Foods and their Weights*, developed by the Dietetics Department of the University of Ghana, Legon, was used to convert the quantities of the foods recorded in the 24-hour recalls into grams. Esha Food Processor® Nutrition Analysis Software was used to analyse the nutrient contents. Results from the analyses of the 24-hour recall were compared to recommended intakes for the population under study, specifically using the Acceptable Macronutrient/Micronutrient Distribution Ranges (AMDR) (IOM, 2000) and this was used to categorize participants as having adequate or inadequate intakes. Participants

whose intakes were less than the AMDR were categorized as having inadequate intakes, those with intakes within the ranges were categorised as having adequate intakes for all nutrients of interest.

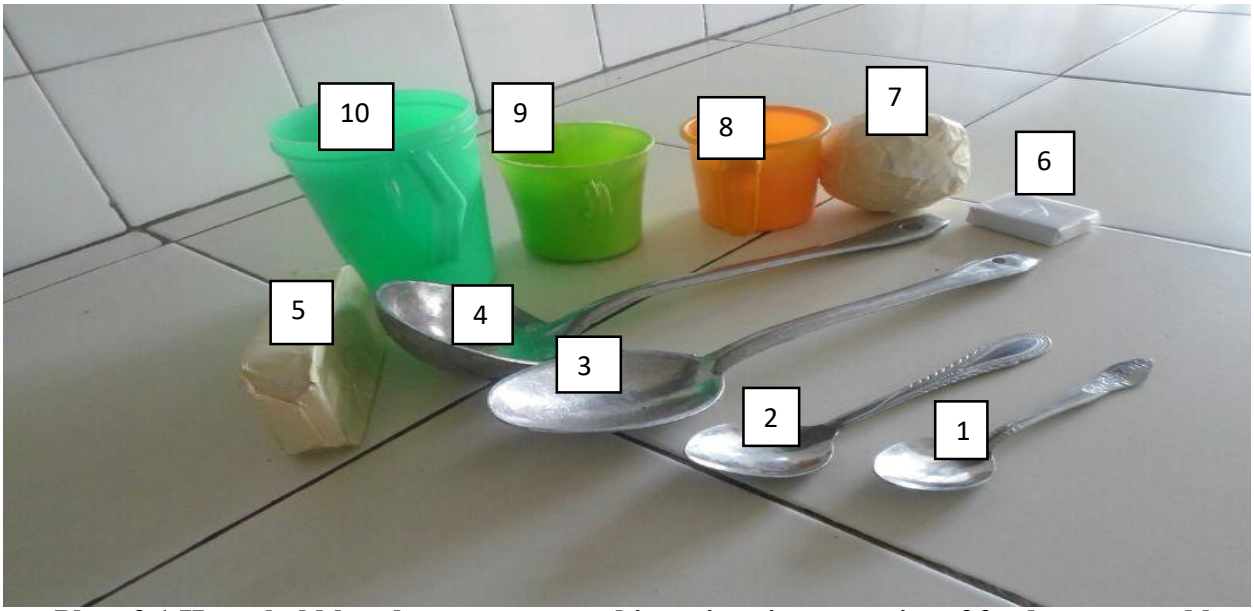


Plate 3.1 Household handy measures used in estimating quantity of foods consumed by respondents

- Label 1 and label 2 – Teaspoon (5ml) and Dessert spoon (10ml) respectively: These were used in estimating or quantifying the quantities of sugar, milk and oil.
- Label 3 – Stewing Spoon/ladle: This was used to estimate quantities of rice and stew consumed by respondents.
- Label 4 – Soup ladle: This was used to estimate the quantities of soups, porridges and gari consumed by subjects.
- Label 5 - Sardine tin: The sardine tin was used to estimate yam, bread and cassava sizes consumed by the participants.
- Label 6 – Match box: The match box was used to quantify respondents' intake of fish, chicken and meat.

- Label 7 – Orange ball: This was used to estimate the quantities of 'akple', 'banku', 'fufu', 'kenkey', 'rice balls', 'tuo zaafi', doughnuts and 'konkonte' consumed by respondents.
- Labels 8, 9 and 10 –200ml, 250ml and 550ml cups respectively: These were used to estimate respondents' intake of porridges and beverages.

3.7.5 Biochemical Assessment

After an 8 – 12 hour overnight fast, 5ml of venous blood sample was collected from the antecubital vein of study participants by a professional phlebotomist into a plain-gel test tube and was allowed to clot. The collected blood samples were centrifuged at 3,500rpm for 3 minutes to obtain serum samples. Serum samples for ALT and AST were kept in empty test tubes while the rest were kept into two heparinized test tubes for vitamin E and zinc analyses. All samples were preserved frozen at below -22^{0}C until it was time to analyse them. Serum zinc, ALT and AST were analysed using an automated analyser while vitamin E was analysed using High Performance Liquid Chromatography (HPLC) Enzyme-linked Immunosorbent Assay (ELISA) sandwich method. The laboratory analyses were carried out at the Volta Regional Hospital Laboratories in Ho and the Molecular Medicine Laboratory, School of Medical Sciences, KNUST in Kumasi.

3.7.5.1 Serum Aspartate aminotransferase (AST)

Principle

This is based on the method of Karmen *et al.,* (1955).

L-Aspartate + α-Ketoglutarate----AST----¾ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H+ ----MDH----¾L-Malate + NAD^++H_2O

The transfer of amino group from L-aspartate to α-Ketoglutarate to produce oxaloacetate and L-glutamate is catalysed by Aspartate aminotransferase (AST). The oxaloacetate goes through reduction with concurrent oxidation of NADH to NAD^+ in the malate dehydrogenase (MDH) catalysed indicator reaction. The subsequent rate of decrease in absorbance at 340nm is directly proportional to the AST activity. To prevent endogenous pyruvate, which is normally present in serum from interfering in the reaction, lactate dehydrogenase (LDH) is added.

Quality Control

With a control sera of known normal and abnormal AST (SGOT) values, the validity of the reaction was monitored. The controls were run daily before AST (SGOT) assays were carried out.

Reagents

Buffer substrate was prepared by mixing100 mmol/L phosphate buffer and 2 mmol/L of 2-oxoglutarate with 100 mmol/L L-asparate.

2. 2, 4 Dinitrophenylhdrazine (DNPH)-1 mmol (200mg)/L in l mol/L HCL.

3. Sodium hydroxide solution 400 mmol (16g)/L.

4. Pyruvate solution – 2 mmol/L (prepared dissolving 22mg of sodium pyruvate in 100ml of distilled water).

Procedure:

Participants' serum samples were taken from storage in a freezer and allowed to thaw. Test tubes were marked and then filled with samples. Standard and blank solutions were prepared. Incubation was done at 37ºC for 60 minutes after which 0.5ml DHPH

was added to each tube. The solution was gently and well mixed and was allowed to stand at room temperature. After 20 minutes, 0.5ml of NaOH was added to each tube. Reading was made at 520nm after 5 minutes using an automated chemical analyser.

3.7.5.2 Serum Alanine aminotransferase (ALT)

Principle

This is based on the 1980 IFCC recommended method (Schumann *et al*., 2002).

$$\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^{+} + H_2O$$

The transfer of the amino group from L-alanine to α-ketoglutarate is catalysed by ALT, causing L-glutamate and pyruvate to be generated. There is a simultaneous oxidation of NADH to NAD^{+} as lactate dehydrogenase catalyses the reduction of pyruvate. The resultant rate of reduction in absorbance is directly proportional to ALT activity.

Quality Control

With a control sera of known normal and abnormal ALT (SGPT) values, the validity of the reaction was monitored. The controls were run daily when ALT (SGPT) assays are carried out.

Reagents:

1. Buffer substrate was prepared by mixing 100 mmol/L phosphate buffer and 2 mmol/L of 2-oxoglutarate with 200 mmol/L-DL alanine.

2. 2,4 Dinitrophenylhdrazine (DNPH)-1 mmol (200 mg)/L in lmol/L HCL.

3. Sodium hydroxide solution 400 mmol (16g)/L.

4. Pyruvate solution – 2 mmol/L (22mg of sodium pyruvate in 100 ml of distilled water).

Procedure:

Participants' serum samples were taken from storage in a freezer and allowed to thaw. Test tubes were marked and then filled with samples. Standard and blank solutions were prepared. Incubation was done at 37ºC for 30 minutes after which 0.5ml DHPH was added to each tube. The solution was gently and well mixed and was allowed to stand at room temperature. After 20 minutes, 0.5ml of NaOH was added to each tube. Reading was made at 520nm after 5 minutes using an automated chemical analyser.

3.7.5.3 Serum Vitamin E

Serum vitamin E was measured using the Human Vitamin E (VE) Enzyme-linked Immunosorbent Assay (ELISA) from Biobase Biotech Ltd. Shandong, China and an Inqaba Biotech Micro Plate Reader Elisa Plate Analyser, Inqaba Biotechnical Industries (Pty) Ltd. Pretoria, South Africa.

Principle

The Sandwich-ELISA method was adopted by the ELISA kit used. A Microelisa strip plate provided in the kit was pre-coated with an antibody specific to vitamin E Standards. A Horseradish Peroxidase (HRP) - conjugated specific for vitamin E was added to each Microelisa strip plate well and incubated. Free components were washed away. The 3, 3', 5, 5'-Tetramethylbenzidine (TMB) substrate solution was added to each well. Only those wells that contain vitamin E and HRP conjugated vitamin E

antibody appeared blue in colour and then turned yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm. The OD value is proportional to the concentration of vitamin E. The concentration of vitamin E in the samples was calculated by comparing the OD of the samples to the curve.

Procedure

- Dilution of Standards: Ten wells were set for standards in Micro Elisa strip plate. In Wells 1 and 2, 50µl standard dilution buffer and 100µl standard solution were mixed well. Wells 3 and 4, were filled with 100µl solution from Wells 1 and 2 respectively. Then 50µl standard dilution buffer were added and mixed well. Fifty microlitres of solution was discarded from Wells 3 and 4. The procedure was repeated by filling the next 2 sets of wells from the immediately preceding ones until wells 9 and 10 were filled. Then 50µl standard dilution buffer was added and mixed well, 50µl solution was discarded from Wells 9 and 10.
- A well was left empty in the Micro Elisa strip plate, as blank control. In sample wells, 40 µl Sample dilution buffer and 10µl sample were added (dilution factor was 5). The serum samples were loaded onto the bottom without touching the wall of the well. Mixing was done properly by gentle shaking.
- Incubation: incubation was done for 30 minutes at 37˚Cwith the plate sealed with closure plate membrane.
- Dilution: dilution was done by washing the concentrated buffer with distilled water 30 times.

- Washing: after carefully peeling off closure plate membrane, it was aspirated and refilled with the wash solution. The solution was discarded after the wash solution rested for 30 seconds. The washing procedures were done 5 times.
- Fifty microlitres (50µl) of HRP-Conjugate reagent was added to each well except the blank control well.
- Incubation and washing were done as described earlier.
- Colouring: Fifty microlitres (50µl) Chromogen Solution A and 50µl Chromogen Solution B were added to each well, mixed by gentle shaking and incubated at 37°C for 15 minutes. Light was avoided during the colouring.
- Termination: Fifty microliters of stop solution was added to each well to terminate the reaction. The colour in the well changed from blue to yellow.
- Absorbance, optical density (OD) was read at 450nm using Microtiter plate Reader. The OD value of the blank control well was set as zero. The assay was carried out within 15 minutes after adding stop solution.

3.7.5.4 Serum Zinc

Zinc test kit (colorimetric) from Biobase Biodustry (Shandong) Co., Ltd, China, was used for the analyses. The URIT 8030 Automatic Chemical Analyser, URIT Group of Companies, China equipment was used for the analyses.

Procedure

Sixty microlitres (60 µL) of serum samples derived from participant's blood were pipetted into test tubes. About 1000 µL of reagent 1 was added and the mixture was incubated in a water bath at 37ºC for 5 minutes. Two hundred and fifty microlitres (250 µL) of reagent 2 was added to the test tube and mixed thoroughly and thereafter incubated in a water bath for 5 minutes. Absorbance was read immediately at 570nm.

3.8 DATA PROCESSING AND ANALYSES

Data entry and analyses were done using the IBM® Statistical Package for Social Sciences (SPSS) version 23. Normality tests were carried on all continuous variables. The Chi-Square ($\chi 2$) goodness-of-fit test and the Chi-Square ($\chi 2$) test of independence were performed on one categorical dichotomous, nominal or ordinal variable and between two categorical variables respectively. The Spearman's correlation was also performed against the null hypothesis of no monotonic association between nonparametric variables. A p value less than 0.05 at 95% confidence interval, was considered statistically significant for analyses.

Continuous variables that were normally distributed were presented as mean and standard deviation and those that were not, were reported as median (minimum and maximum). Categorical variables were presented as frequencies, proportions and percentages. Results were presented in tables, pie charts and graphs and interpretations of findings were made as possible.

3.9 ETHICAL CONSIDERATION

The Committee on Human Research, Publications and Ethics (CHRPE), School of Medical Sciences (SMS – KNUST) gave ethical approval with reference, Ref: CHRPE /AP/170/17 (see Annex 6 in appendices) for the conduct of this study. Permission was also given by the Management of the Volta Regional Hospital with reference, My Ref. No. VRH/5/14 also shown in Annex 7 of appendices, for their facilities to be used for the study. Informed consent was obtained from the participants of the study.

CHAPTER FOUR

RESULTS

4.1 BACKGROUND OF STUDY PARTICIPANTS

4.1.1 Socio-Demographic Characteristics of Participants

The socio-demographic background of the study participants are presented in Table 4.1. A total of one hundred and three (103) took part in the study. Out of this number, (77) 74.80% were females and 26 (25.20%) were males. Most of the participants (74.80%) have just basic level education and majority of them (81.60%) were self-employed.

Table 4.1: Socio-demographic characteristics of study participants stratified by gender

Characteristic	Male (%)	Female (%)	Total (%)
Ethnicity			
Ewe	24 (92.3)	73 (94.8)	97 (94.2)
Akan	1 (3.8)	3 (3.9)	4 (3.9)
Other	1 (3.8)	1 (1.3)	2 (1.9)
Total	26 (25.2)	77 (74.8)	103 (100.0)
Marital status			
Single	4 (15.4)	13 (16.9)	17 (16.5)
Married	14 (53.8)	32 (41.6)	46 (44.7)
Divorced	3 (11.5)	15 (19.5)	18 (17.5)
Widowed	5 (19.2)	17 (22.1)	22 (21.4)
Educational background			
No formal education	2 (7.7)	6 (7.8)	8 (7.8)
Basic	17 (65.4)	60 (77.9)	77 (74.8)
Secondary	5 (19.2)	8 (10.4)	13 (12.6)
Tertiary	2 (7.7)	3 (3.9)	5 (4.9)
Occupation			
Civil servant	5 (19.2)	4 (5.2)	9 (8.7)
Self-employed	15 (57.7)	69 (89.6)	84 (81.6)
Unemployed	2 (7.7)	4 (5.2)	6 (5.8)
Others	4 (15.4)	0 (0.0)	4 (3.9)
Age	**Median (max–min)**		
	51 (24–88)	46 (24–76)	

Data is presented as absolute values with corresponding percentages in parentheses, median (maximum minimum)

4.1.2 HIV Type of Participants

Table 4.2 shows the type of HIV that infected participants. The prominent type of HIV that infected the participants was type 1 (94.2%) and this trend is similar between both genders. While 15.4% of the males had HIV type 1 and 2 co-infection, it was only 1.3% in the case of female participants.

Table 4.1 HIV type among participants

HIV type	HIV 1 (%)	HIV 2 (%)	HIV 1 & 2 (%)	Total (%)	*p* – value
Male	22 (84.6)	0 (0.0)	4 (15.4)	26 (100.0)	0.000*
Female	75 (97.4)	1 (1.3)	1 (1.3)	77 (100.0)	0.000*
Total	97 (94.2)	1 (1.0)	5 (4.8)	103 (100.0)	0.000*

Data is presented as actual values with percentages in parentheses. **p-value* is significant at $p < 0.05$.

4.1.3 Duration of HIV infection and HAART use among participants

The Figure 4.1 illustrates the duration since participants were diagnosed with HIV and when HAART was initiated in years. Sixty-one (61) participants representing 59.2% were diagnosed less than 5 years ago. Participants who were diagnosed with the virus between 5 and 10 years prior to the collection of this data constituted 36.9% of the study population. A little over half of participants, 55 (53.4%) were on HAART for less than 5 years at the time of data collection. Forty four (44) participants representing 43% were also on treatment that spanned from 5 to 10 years while 4.2% were on the treatment for 10 years and above.

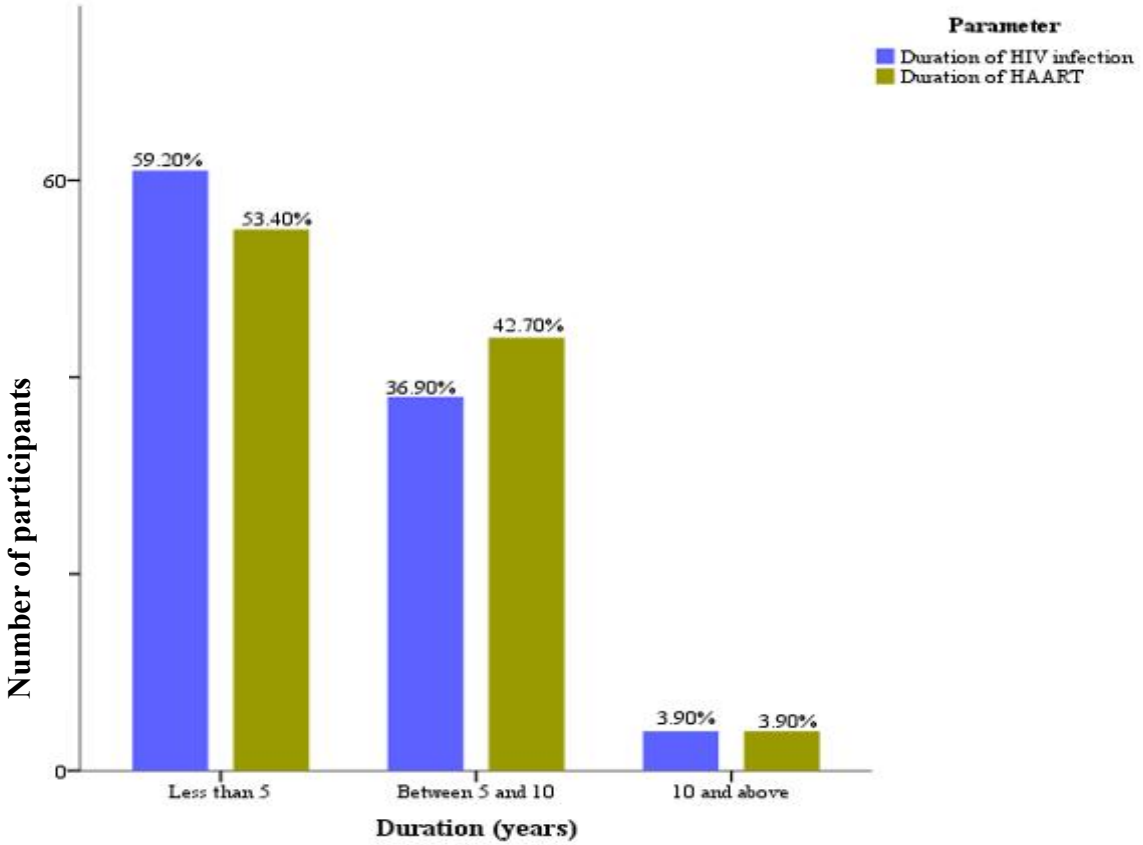


Figure 4.1 Distribution of HIV infection and HAART use among participants

4.1.4 HAART use and distribution among participants

Most of the participants (45.6%) have been on TDF-3TC-EFV HAART combination in the past 6 months to the time of data collection. Only one person was taking AZT-3TC-LPV/r combination in the past 6 months preceding data collection. Almost an equal proportion (26.2% and 27.2%) of participants, were taking AZT-3TC-NVP and AZT-3TC-EFV combinations respectively. Table 4.3 summarises the information across gender.

Table 4.3 HAART combinations of participants for the past six (6) months

HAART combination	Male (%)	Female (%)	Total (%)
TDF+3TC+EFV	11 (10.7)	36 (34.9)	47 (45.6)
AZT+3TC+NVP	7 (6.8)	20 (19.4)	27 (26.2)
AZT+3TC+EFV	7 (6.8)	21 (20.4)	28 (27.2)
AZT+3TC+LPV/r	1 (1.0)	0 (0.0)	1 (1.0)
Total	26 (25.3)	77 (74.7)	103 (100)

Data is presented as absolute values and percentages. TDF-Tenofovir, AZT- Zidovudine, 3TC-Lamivudine, NVP-Nevirapine, LPV/r-Ritonavir boosted Lopinavir

4.2 ANTHROPOMETRY AND NUTRITIONAL STATUS

4.2.1 Anthropometric characteristics of participants

The anthropometric data are presented in Table 4.4. Except for height, there was no gender difference of the anthropometric parameters of the participants.

Table 4.4: Anthropometric characteristics of participants across gender

Anthropometric characteristics	Male (n=26)	Female (n=77)	Total (n=103)	*p*-value
Weight(Kg)	61.01±12.58	61.25±14.32	61.19±13.84	0.935
Height(m)	1.65±0.80	1.59±0.06	1.60±0.74	0.002*
BMI(Kg/m^2)	22.38±4.14	24.16±5.13	23.71±4.95	0.081

Data is presented as mean ± standard deviation.*p-value is significant at $p < 0.05$. BMI – Body Mass Index

The nutritional status of the participants was determined using their body mass index BMI. The mean BMI for the participants (Table 4.4), was 23.71±4.95kg/m^2 making most of them (55.3%) to fall within the normal BMI range. The proportion of study subjects that were underweight was 11.7% while the same proportion (11.7%), was

obese. Half of the males (50.0%) and 57.1% of the females, recorded normal BMI (Table 4.5).

Table 4.5 Anthropometric distribution of participants

Gender	BMI				Total (%)	*p*-value
	Underweight (%)	Normal (%)	Overweight (%)	Obese (%)		
Male	6 (23.1)	13 (50.0)	5 (19.2)	2 (7.7)	26 (100.0)	0.019*
Female	6 (7.8)	44 (57.1)	17 (22.1)	10 (13.0)	77 (100.0)	0.000*
Total	12 (11.7)	57 (55.3)	22 (21.4)	12 (11.7)	103 (100.0)	0.000*

Data is presented as actual values with percentages in parentheses. **p-value* is significant at $p < 0.05$.

4.2.2 Anthropometric distribution by duration of HIV infection and HAART use

On average, participants who were undernourished (BMI < 18.5Kg/m^2) recorded the highest duration of HIV infection and HAART use (5.58 and 5.69 years respectively). Participants with normal BMI or who were overweight recorded nearly same duration of HAART (4.47 versus 4.41 years respectively) while obese individuals recorded slightly higher duration of HIV infection and HAART use than both normal and overweight individuals (Figure 4.2).

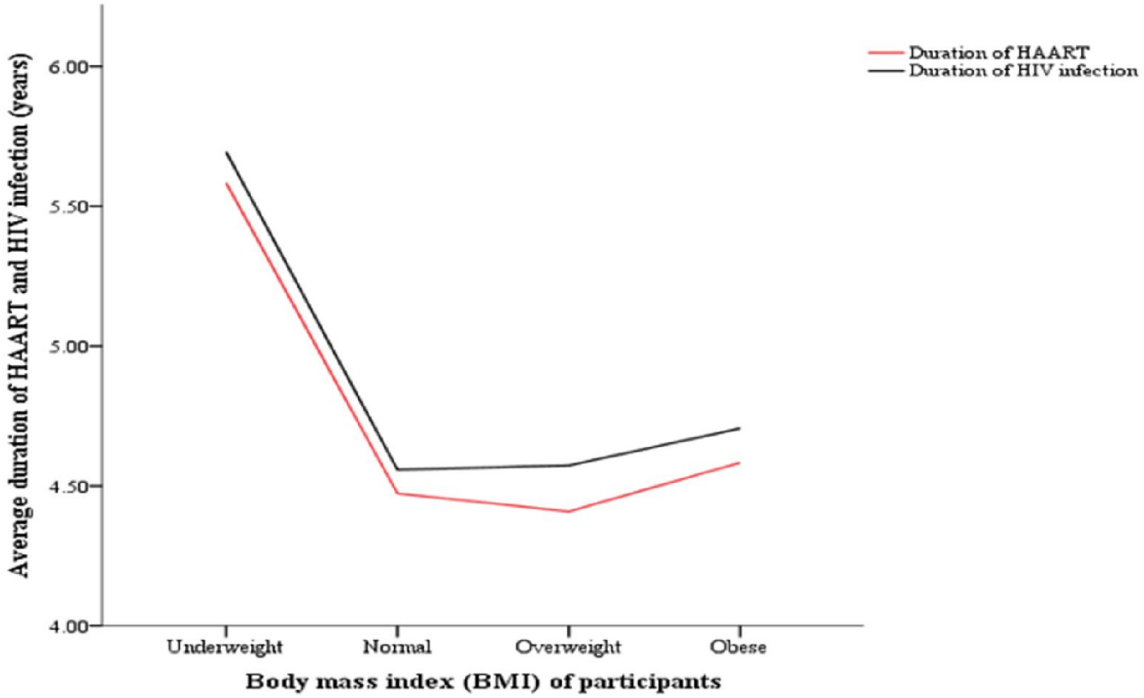


Figure 4.2: Average duration of HAART and HIV infection by BMI of participant

4.3 BIOCHEMICAL PARAMETERS OF PARTICIPANTS

4.3.1 Serum Zinc

Majority (91.3%) of the study participants based on Johns Hopkins Medical Laboratories reference values had normal serum levels of zinc. Table 4.6 shows the details.

Table 4.6 Serum zinc levels of participants

Reference Range	n = 103	$\chi 2$	*p*-value
0 – 9.2 (µmoI/L)	9 (8.7%)	70.2	0.000*
9.2 – 19.9 (µmoI/L)	94 (91.3%)		

Data is presented as absolute values (n) with corresponding percentages in parentheses. a - based on Johns Hopkins Medical Laboratories reference values (9.2 – 19.9 (µmoI/L)). **p*-value is significant at $p < 0.05$.

4.3.2 Serum vitamin E

The pie chart presented in Figure 4.3 shows the distribution of serum levels of vitamin E of the participants. Majority (82.5%) of the participants recorded less than 12µmol/L compared to 17.5% of them who had serum vitamin E levels of 12µmol/L and above.

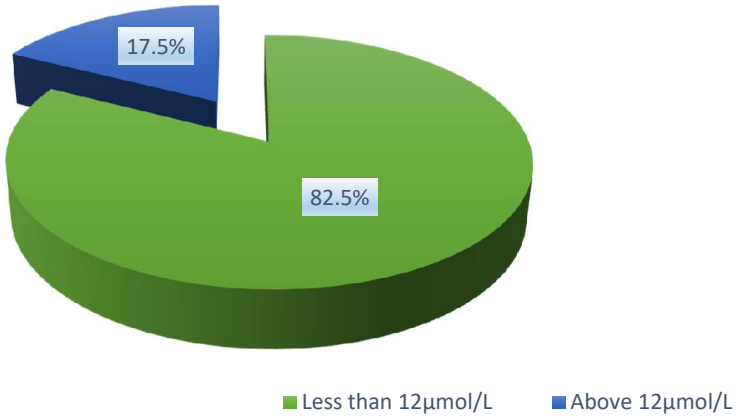


Figure 4.3: Serum vitamin E levels of participants

4.3.1 Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

There was a median decrease in serum levels among the participants after the initiation of HAART (from 17.0 IU/L to 13.0 IU/L). The opposite however holds for serum AST levels of participants which increased post-HAART from 22.0 IU/L to 30.4 IU/L. The details are presented in Table 4.7.

Table 4.7: Serum AST and ALT (pre and post HAART)

Liver enzyme	Pre-HAART median (min-max)	Post-HAART median (min-max)	*p*-value
ALT(IU/L)	17.0 (6.0-61.0)	13.0 (4.20-70.8)	0.015*
AST(IU/L)	22.0 (11.0-57.0)	30.4 (7.22-99.5)	0.000*

Data is presented as median (minimum-maximum). AST – Aspartate Transaminase, ALT-Alanine Transaminase. **p-value* is significant at $p < 0.05$.

4.4 DIETARY INTAKE OF HIV INFECTED INDIVIDUALS

4.4.1 Nutrients Intake among participants

Table 4.8 shows results of nutrients analysed from the actual dietary intakes of the participants. Their calorie and dietary fat intakes were below requirement. Participants also recorded intakes of zinc and vitamin A within acceptable recommended ranges. The intake of dietary vitamins C, D and E were however below those recommended.

Table 4.8: Pattern of nutrient intake of study participants

Nutrients	Nutrient intake of participants Median (min – max)	AMDR	*p*-value
Calories (Kcal)	1680 (384-4688)	2030	0.004*
Macronutrients			
Carbohydrate (g)	254 (76-836)	228 – 280	0.181
Fat(g)	54 (8-156)	56 – 79	0.000*
Protein(g)	56 (4-129)	50 – 76	0.000*
Micronutrients			
Zinc (mg)	8.47 (2-28)	8 – 11	0.033*
Vitamin A (µg)	828 (0-350)	700 – 900	0.002*
Vitamin C (mg)	54 (0-335)	75 – 90	0.009*
Vitamin D (µg)	2 (0-89)	15 – 20	0.000*
Vitamin E (mg)	3 (0-19)	15	0.000*

Data is presented as median with minimum and maximum values in parentheses. AMDR – Acceptable Macro/Micro-nutrient Distribution Ranges/Values for adults (IOM, 2000). Chi square goodness-of-fit test performed. **p-value* is significant at $p < 0.05$.

4.4.2 Consumption of antioxidant micronutrient based foods among participants

Figure 4.4 shows the pattern of consumption of antioxidant micronutrient rich foods by participants. Vitamin A foods include palm oil, carrot, tomato, pawpaw, banana and lettuce. Majority (59.2%) of the participants consumed these foods at least once a week while 38.8% of them occasionally consumed these foods.

The vitamin C rich foods included citrus fruits, pineapple, watermelon, apple and guava. A large number of the participants reported occasional consumption of these

foods. Only 12.60% of them consumed foods containing vitamin C at least once a week while 4.90% of them reported no intakes at all of these foods.

The consumption patterns of zinc rich food such as oysters, salmon, tuna, mutton and tiger nuts as well as vitamin E rich foods such as herrings, plant oil and dandelion among participants was also determined. Most of the participants occasionally consumed vitamin E (61.17%) and zinc (67.96 %) rich foods. Only a few of them reported a frequency of at least once per week of consumption of vitamin E (33.01%) and zinc (24.27%) rich foods. The proportion of participants who did not consume any of the vitamin E and zinc rich foods used in this study in the past 6 months prior to data collection are 5.82% and 7.77% respectively.

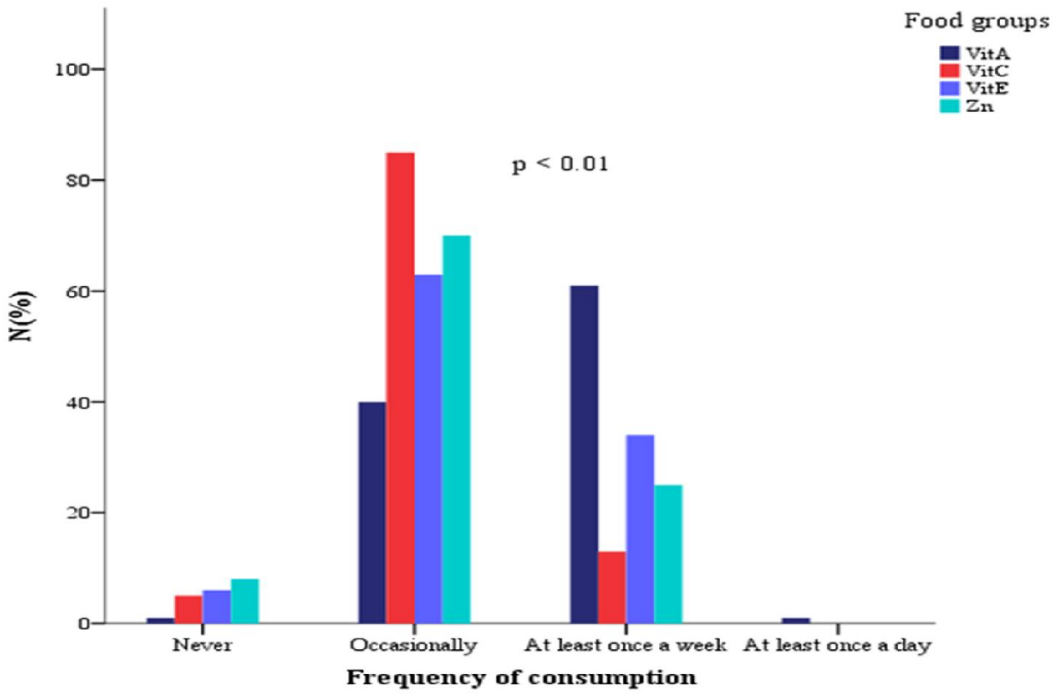


Figure 4.4: Participants' consumption patterns of antioxidant rich foods

4.4.3 Alcohol intake, herbal medicine use and smoking among participants

Only 9 (8.7%) participants of the study population consumed at least one type of alcoholic drink. No participant consumed herbal medicine or made use of tobacco products and recreational drugs. These results are presented in table 4.9.

Table 4.9: Consumption of alcohol, herbal medicine and substance abuse

ACTIVITY	YES (%)	NO (%)
Alcohol consumption	9 (8.7)	94 (91.3)
Intake of herbal medicine	0 (0)	103 (100.0)
Use of tobacco products	0 (0)	103 (100.0)
Use of recreational drugs	0 (0)	103 (100.0)

Data is presented as actual values with corresponding percentages in parentheses.

4.4.4 Dietary zinc Intake and serum zinc levels of participants

Figure 4.5 shows the correlation between dietary zinc intake from a 24-hour dietary recall and serum zinc levels of study participants. The 0.90% of the variability of serum zinc levels of the participants being accounted for by their dietary zinc intake was not statistically significant. Serum zinc level of the study participants was independent of their dietary zinc intake as measured from their 24-hour dietary recall.

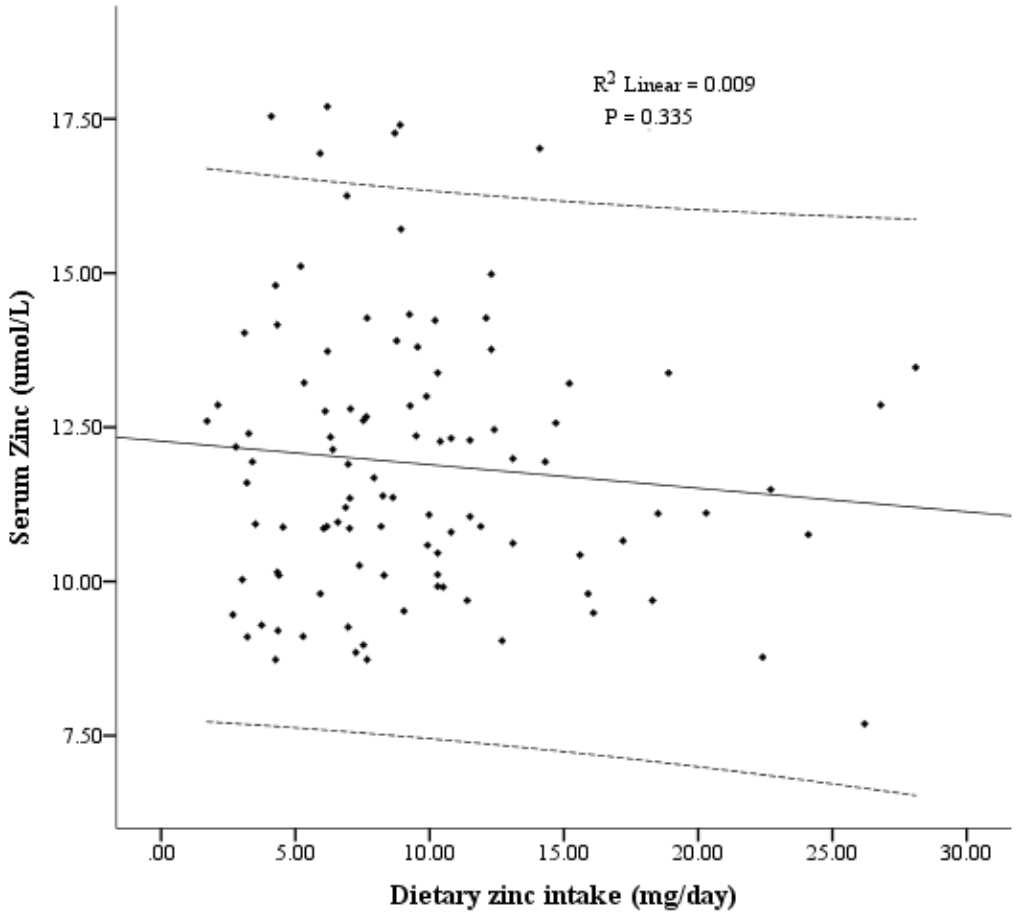


Figure 4.5: Dietary zinc intake and serum zinc levels of participants

4.4.5 Dietary vitamin E intake and serum vitamin E levels of participants

Table 4.10 presents a correlation between dietary vitamin E intake and serum vitamin E levels. There is no association between the two parameters (p=0.993).

Table 4.10: Dietary vitamin E and serum vitamin E levels of participants

	Serum Vitamin E (µmol/L) r^2 **(*p*-value)**
Total vitamin E intake (mg)	-0.001 (0.993)

Data is presented as r^2 (p-value). r^2-coefficient of determination. *p-value* is significant at $p < 0.05$.

4.5 SERUM VITAMIN E AND ZINC LEVELS AND THEIR RELATIONSHIP WITH DURATION OF HIV INFECTION AND ON HAART

Table 4.11 presents correlation analyses between serum levels of vitamin E and zinc against duration of HIV infection and duration on HAART. Serum levels of vitamin E was unaffected by participants' duration on HAART. Furthermore, duration of HIV infection which was calculated using the time since diagnoses of HIV infection also did not have any significant effects on participants' vitamin E levels in the serum.

Even though the serum levels of zinc of the participants were significantly affected by duration on HAART and HIV infection, only 5.3% and 4.8% of the variability of serum levels of zinc of the participants could be accounted for by duration of HAART and HIV infection respectively.

Table 4.11: Effects of duration of HAART and HIV infection on serum zinc and vitamin E of participants

Parameter	**HAART duration (years) r^2**	**(*p*-value)**	**Duration of HIV infection (years) r^2**	**(*p*-value)**
Serum zinc (µmol/L)	0.053	0.021*	0.048	0.027*
Serum vitamin E (µmol/L)	0.001	0.313	0.010	0.405

Data is presented as r^2 (*p*-value). r^2-coefficient of determination. **p-value* is significant at $p < 0.05$.

4.6 SERUM VITAMIN E AND ZINC LEVELS AND BMI DISTRIBUTION AMONG PLWH

Figure 4.6 shows the line chart of participant's serum levels of vitamin E and zinc (µmol/L) across their body mass index (BMI). Obese individuals recorded the lowest mean serum levels of vitamin E (8.41µmol/L) while participants with BMI values less than 18.5Kg/m^2 recorded the highest serum levels of Vitamin E (9.28µmol/L). Even so, the serum levels of the nutrient of this category of participants were below normal (12µmol/L) based on the cut off by Food and Nutrition Board, Institute of Medicine (2000). Participants who had either normal or overweight BMI recorded nearly similar serum levels of vitamin E that were also below normal (8.91 and 8.94µmol/L respectively). Participants whose BMI fell below 18.5Kg/m^2 recorded the highest serum zinc levels (12.18µmol/L). Participants with BMI values from 25 to 29.9 Kg/m^2 however, recorded the lowest serum zinc levels (11.98µmol/L). Even so, the mean serum levels of vitamin E and zinc across BMI of study participants did not differ significantly from zero (p= 0.15).

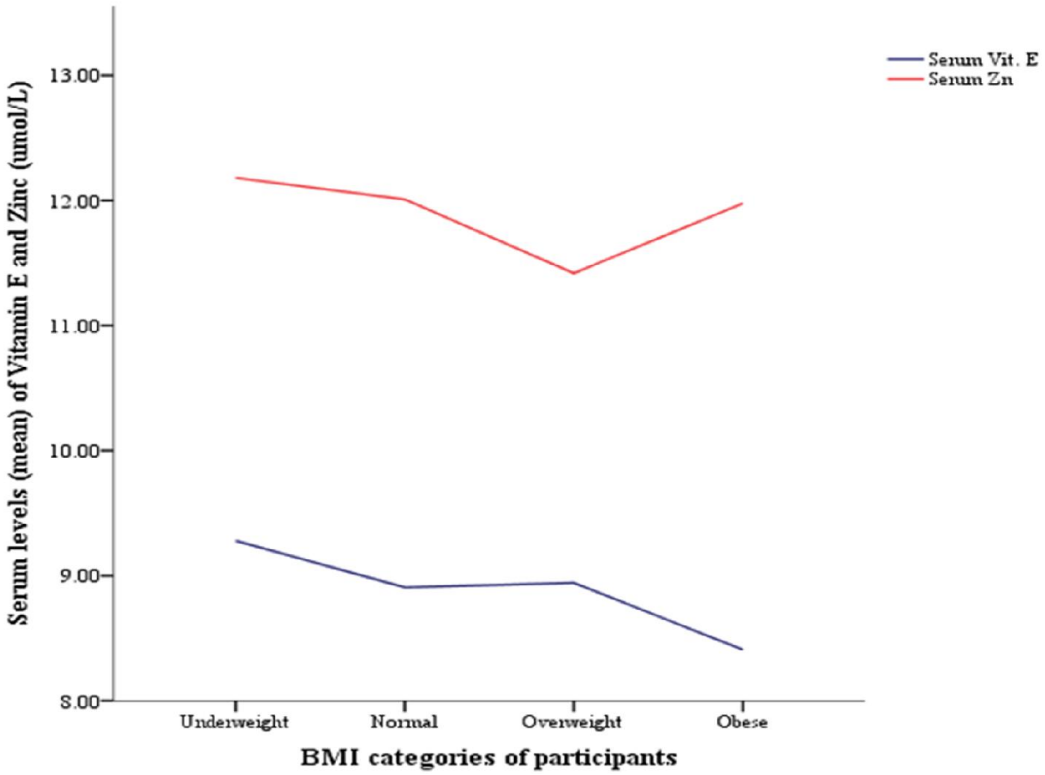


Figure 4.6: Mean serum levels of Zinc and Vitamin E by BMI categories of participants

4.7 RELATIONSHIP BETWEEN SERUM VITAMIN E AND ZINC AND SERUM LIVER ENZYMES

Presented in Table 4.12 is a correlation between AST and ALT and serum vitamin E and zinc levels of participants. There were positive correlations between all the parameters but they were not significant.

Table 4.12: Liver enzymes and serum vitamin E and zinc

LIVER ENZYMES	SERUM VITAMIN E r^2	*p*-value	SERUM ZINC r^2	*p*-value
AST (IU/L)	0.072	0.473	0.009	0.925
ALT (IU/L)	0.052	0.607	0.073	0.470

Data is presented as r^2 (*p*-value). r^2-coefficient of determination. *p-value* is significant at $p < 0.05$. AST-Aspartate Transaminase, ALT- Alanine Transaminase.

CHAPTER FIVE

DISCUSSION

The main objective of this study was to assess antioxidant micronutrient intake and its implication on serum levels as well as effects on liver function among HIV positive individuals undergoing antiretroviral therapy. In this study, 103 HIV infected adults comprising of 25.2% males and 74.8% females who are on Highly Active Antiretroviral Therapy (HAART) at the Volta Regional Hospital in Ho in Ghana were randomly selected. The male to female ratio of participants in this study corresponds with the fact that, there are more females (59%) than males (41%) living with HIV in Ghana (Ghana AIDS Commission, 2015). Globally, the UNAIDS status report (2016) estimated that there were 51% females and 49% males living with HIV as at the end of 2015. The report indicated that women made up 56% of all new HIV infection in Africa. Harmful gender norms and inequalities, insufficient access to education and sexual reproductive health services, violence and poverty were among reasons cited to increased risk of HIV infection among adolescent females and women (UNAIDS, 2016).

A large number of the participants (74.8%) in the study had just basic level (primary to junior high school) as their highest educational attainment and majority of the participants (82%) were self-employed (Table 4.1). This outcome corroborates with that of the Ghana AIDS Commission (2006) report which stated that individuals with primary and secondary education have the larger prevalence of HIV infection. Having a low educational background will imply inadequate skills, technical knowledge and the ability to get a well-paying job, hence less income and reduced ability to acquire nutritious food and healthcare (Ghana AIDS Commission, 2006).

In view of the possible physiological and metabolic consequences of HIV infection, it has been recommended that caloric intake should be increased than in the general population. Even though there is no evidence to support increasing fat, protein and micronutrients intakes, recommendations for the intake of balanced diet from a variety of food groups by PLWH have been made (FANTA, 2004; WHO, 2004).

Dietary assessment results of participants in this study revealed a lower caloric and fat intake (Table 4.8). This may be attributed to underreporting by participants due to the reliance on memory to recall previous dietary intakes. This finding corroborates well with those reported by Juma *et al*. (2016) in Kenya where they documented low energy and fat intake for nearly half of the population (120 HIV infected individuals) studied. It is however in contrast with what was found by a study conducted by Hendricks *et al*. (2006) in the United States (US), aimed at describing obesity among 321 HIV infected adults. The study found high caloric and fat intakes among the cohort. Similar findings were made by Klassen and Goff (2013) who reported higher than recommended saturated fat intakes among their cohort in the UK. Energy requirements among people infected with HIV have been shown to vary greatly, with obese people possessing less metabolically active tissues hence, lower energy per kilogram weight requirements. High intakes of saturated fats is associated with increased risk of developing cardiovascular diseases and contribute to abnormalities in lipid metabolism during HAART.

This study found adequate consumption of proteins. Adequate protein intakes among the HIV infected population have been documented by Giudici *et al*. (2013). This is however contradicted in a research carried out in Kenya by Juma *et al*., (2016) where low intakes of proteins were realised among the participants. Even though an increase in protein intake in HIV infection has no evident benefit, it is also important to

maintain adequate intakes in this population just as the general population. This will help to maintain nitrogen stores which are being constantly lost in HIV and associated opportunistic infections (WHO, 2005; WHO, 2004; FANTA, 2004; Keithley *et al*., 2000).

The importance of micronutrients to the human body and especially in immunity and metabolism cannot be overstated (Tang *et al*., 2005). The risk of HIV positive individuals becoming deficient in these micronutrients is high due to infection, inadequate intake and malabsorption (Garcia-Prats *et al*., 2010). In this study attention was given to certain micronutrients of interest due to their function in antioxidation. The dietary intakes of some of these nutrients among the participant and the serum levels of two of them (vitamin E and zinc) were determined.

Results from this study showed that vitamin A and zinc intakes among the participants were up to the recommended intakes (Table 4.8). The consumption of vitamins C, D and E did not meet the recommended intakes. Vitamin A intake among participants was through food consumption as more than half of the participants consumed foods rich in vitamin A at least once in a week (Figure 4.4). Participants in this study were chosen based on the fact that they were not on any supplementation regimen of the micronutrients understudy since the focus was on dietary sources. Adequate vitamin A and inadequate vitamin C intakes were reported by Juma *et al*., (2016) who attributed the adequacy in the intake of vitamin A in their study to monthly supplementation. Vitamin A is important in the proper functioning of the immune system, and the maintenace of cellular intergrity (Garcia-Prats *et al*., 2010).

The inadequate intake of vitamin C by participants in this study can be attributed to low consumption rates of vitamin C rich foods as most participants consumed these foods just ocassionally (Figure 4.4). Vitamin C is an antioxidant micronutrient that is very

essential in the prevention of oxidative stress in humans (Padayatty *et al*., 2003). More specifically vitamin C as electron donor donates electrons to oxidixed compounds thereby reducing them and making them become stable. Vitamin C helps in the conversion of alpha tocophoroxyl radical, generated when alpha tocopherol (vitamin E) interacts with low density lipoproteins back to alpha tocopherol (Neužil *et al*., 2005). Adequate vitamin C intake on a daily basis is therefore important to PLWH who are known to be under oxidative stress and also because the vitamin is water soluble and is easily lost from the body. In their study, Stephensen *et al*. (2006) found lower serum vitamin C levels in HIV infected individuals compared to their HIV negative counterparts. They also found lower plasma ascorbate levels and suggested that vitamin C requirements in PLWH are greater than HIV negative individuals.

Another micronutrient of interest in this study is vitamin E, a fat soluble vitamin that plays antioxidation roles in the human body. Due to its role in oxidative reaction chain breaking, it has received much research attention especially among HIV infected individuals. In this study, both dietary intakes and serum levels of the vitamin were assessed. Results from this assessments indicated that both the intake and serum levels of the vitamin E were low among the participants. The median vitamin E intake was very low. About 83% of the participants recorded less than 12µmol/L serum vitamin E levels (Figure 4.3) and according to the Insttitute of Medicine (IOM, 2000), they were deficient in the nutrient. Low dietary vitamin E intake and serum levels have been documented in earlier studies (Obuseh *et al*., 2011; Stephensen *et al*., 2006, Moshfegh *et al*., 2005; Maras *et al*., 2004). About 73% of HIV negative and 88% of HIV positive individuals (total population 147 and 158 respectively) were found to be deficient in vitamin E in a study carried out in Ghana (Obuseh *et al*., 2011). Both low dietary intake and serum levels of vitamin E have been described by Stephensen *et al*. (2006) among

young adults living with HIV. The deficiency of vitamin E is seldom seen in adults but more frequently seen in children apparently due to limited stores and also because children are still growing (Traber, 2014). The population being studied is however unique from the general population. The high proportion of participants with low serum levels of vitamin E therefore calls for concern. It is however, generally known that low biochemical indices of individual vitamins are less prevalent in HIV negative people than HIV infected individuals (Beach *et al*., 1992; Baum *et al*., 1991). In this study, intake of vitamin E was inadequate and may be a reason for the high deficiency rate since the intake depends on the diet (Moshfegh *et al*., 2005). Studies have however revealed that, dietary α-tocopherol intakes do not correlate very highly with circulating α-tocopherol concentrations (Traber, 2014; Fares *et al*., 2011; Kabagambe *et al*., 2005; Laryea *et al*., 1988). This study found results that are in consonance with this previous findings because serum levels of vitamin E of participants did not correlate with their dietary intakes ($p = 0.993$) (Table 4.10).

As an antioxidant, serum vitamin E levels can also reduce as a result of HIV and HAART induced oxidative activity in which case more of this vitamin will be required to prevent oxidative stress. This was however, not supported by data from this study and therefore requires futher investigation as both duration since diagnoses of HIV infection, and HAART use were not associated with serum vitamin E levels (Table 4.11). Apart from causing neurologic disorder, vitamin E deficiency also causes muscle deterioration and this may lead to death (Traber, 2014). In HIV infection, vitamin E deficiency has been associated with hightened wasting levels, viral load and oxidative stress (Beck, 2007). Low serum vitamin E levels found in this study suggests that the participants are at increased risk of suffering oxidative stress and its effects.

Zinc still remains a vital mineral for human health due to its role as a co-factor for over 2000 transcription factors and 300 enzymes as well as an essential mediator of cellular signalling (Roshanravan *et al*., 2015; Jurowski *et al*., 2014). Even though it is not an antioxidant, it functions in protecting cells against oxidative stress. It is thus considered a pro-antioxidant since it protects cells against detrimental effects of oxygen radicals produced through immune activation (Maret, 2006).

Majority of the participants had their serum zinc levels falling within the reference range adopted in this study (Table 4.6). This can be attributed to the recorded adequate dietary intakes among the study population. In addition, the good serum zinc levels could also be attributed to the adequate protein intake of the participants (Table 4.8) since proteins are good zinc sources according to Mahan and Raymond, (2016). The results of this study however contradicts these earlier findings as zinc deficiency has been described as prevalent among HIV infected people (Sneij *et al*., 2016; Baum *et al*., 2010; Wand *et al*., 2007; Baum *et al*., 2003; Visser *et al*., 2003).

Dietary zinc intake did not have significant association with serum levels in this study (Figure 4.5). A similar observation was made by Sneij *et al*. (2016) who found no relationship between zinc intake and plasma zinc levels when an assessment of plasma zinc status of HIV infected individuals who either have hyperglycaemia or normoglycaemia was done. These results are however contrary to what Baum *et al*. (2003) reported in their study when they assessed the serum zinc status of HIV-1 infected people who use illicit drugs. They demonstrated that there was a dose effect of dietary zinc intake on plasma zinc. Zinc supplementation at nutritional levels has been shown to delay immunological failure and decrease diarrhoea over time in patients with poor viral control (Baum *et al*., 2010).

In spite of these positive findings about zinc in this study, there are concerns that excess serum zinc enhances HIV replication and consequently increase viral load and reduce survival. Caution should therefore be taken, taking into consideration especially the dietary intakes of the individuals when implementing supplementation programmes in order to prevent the negative associations of excess zinc intakes.

Malnutrition is associated with HIV infection. This used to mainly be a unilateral problem of undernutrition and wasting of HIV infected individuals probably due to an overall increase in demand for energy as a result of the stress from the infection; and a reduction in intake of energy and nutrients caused by symptoms of opportunistic infections.

In recent times and in the advent of HAART, the trend of malnutrition in HIV infected individuals is gradually changing. There is currently the emergence of both underweight, overweight and obesity among the HIV positive population. This is not different in this study as there was the prevalence of underweight and overweight/obesity among the participants (Table 4.5). Several studies have reported this trend (Takarinda *et al*., 2017: Juma *et al*., 2016).

Results from this study revealed that, 21.40% were overweight and 11.70% were obese (Table 4.5). More women were obese than men and this could depict general trends but could also be due to the wide disparity in the number of women in the study compared to men. Overweight and obesity have been described among PLWHs in a number of studies (Takarinda *et al*., 2017; Banwat, 2013; Hendricks *et al*., 2006; Amorosa *et al*., 2005; Hodgson *et al*., 2001; Shevitz and Knox, 2001). In a study to assess the malnutrition status and its associated factors among PLHWs in Zimbabwe (Takarinda *et al*., 2017), it was found that, 18.4% and 8.0% of the participants were overweight and obese respectively. Obesity is known to have a strong association with numerous

major health risk factors and it has been well accepted that obesity among the general population is linked with health complications such as diabetes, some cancers, cardiovascular disease, and increased mortality (Mokdad *et al*., 2003). Coupled with HIV infection, obesity and its consequences will compound the health challenges that PLWH face.

This study found that participants who have been infected with HIV and have been on HAART for more than 5 years were underweight (Figure 4.2). It therefore suggests that, long term HIV infection and HAART use may reduce BMI. This may be as a result of the increased energy and nutrient requirement in HIV infection and ARV intake and may manifest when intakes are not meeting the requirements.

Majority (55.3%) of the participants in the study had normal BMI (Table 4.5). This could possibly be due to the fact that majority of the participants acquired the infection less than five years ago, had sought for early treatment, and have also been on this treatment for less than five years. (Figure 4.1). This supports the earlier finding that suggests that, long term HIV infection and HAART use negatively affects BMI.

In this study it was found that, obese individuals recorded the lowest serum levels of vitamin E while participants whose BMI fell below the lower normal cut-off had higher vitamin E levels in their serum (Figure 4.6). Findings by Murer *et al*. (2014) led to the conclusion that, obesity does not necessarily mean that vitamin E status is adequate for normal liver functions especially as oxidative stress is high in obese people. It was expected that serum vitamin E will be high in the obese participants due to the fact that they have more circulating lipids that will carry this fat soluble vitamin (Traber, 2014). This calls for the need to understudy the micronutrient status of overweight and obese individuals so as to give a better explanation to this finding. It is however, not easy to interpret circulating α-tocopherol and also worthwhile to know that abnormal

lipoprotein metabolism does not necessarily mean an increased delivery of α-tocopherol to tissues (Traber, 2014).

Some ARVs are associated with excess fat deposition at various parts in the body. When this occurs, it will definitely affect BMI by increasing fat mass hence body weight. Protease inhibitors such ritonavir, has been tagged with this effect. Results from this study however showed that, only one participant is on such an ARV combination that has a protease inhibitor. This suggests that, the high prevalence of overweight and obesity in this study may not be as a result of the ARVs but other causes may be involved.

The determination of serum levels of numerous enzymes including liver enzymes is of great diagnostic importance. The presence of these enzymes in the serum and especially above certain levels indicates a potential cellular or tissue damage making the enzymes to leak alongside other cellular components in to the blood (Srivastava and Chosdol, 2007).

Serum AST and ALT levels assessments are part of a battery of tests that are performed as liver function tests. Elevation of AST and ALT in the serum have been associated with HAART use. Several, if not all antiretroviral drugs are known to cause hepatotoxicity (Sulkowski, 2004). For example the NNRTI nevirapine and efavirenz and most protease inhibitors have been implicated in the causation of hepatic injuries (Sulkowski *et al*., 2002; Sanne, 2000; Centers for Disease Control and Prevention, 2001). A key but not conclusive indicator of hepatic injury is the raising of serum levels of the transaminases, AST and ALT. The definition of liver injury has been outlined by the AIDS Clinical Trial Group (AIDS Clinical Trial Group, 1996).

This study compared the median serum levels of AST and ALT before the initiation of HAART and at the time of collection of data from participants. Results show that there

was a significant increase in serum AST levels with a significant decrease in serum ALT levels after the initiation of HAART. Both median values however, were within the normal range (Table 4.7). The contrasting action in change in these two liver enzymes observed in this study is compared to what Abubakar *et al*. (2014) realised among HIV positive individuals when they assessed the effects of HIV and ARVs on the liver enzymes and CD4+ T- cells in HAART naïve and HAART experienced HIV infected individuals. From their study they observed that, AST and ALT levels were significantly lower in the HIV infected HAART experienced group and significantly higher in the HIV infected HAART naïve group. They concluded that, the elevation in serum liver enzymes was as a result of HIV infection.

Clinical studies have shown that grade 3 and grade 4 hepatotoxicity is seen in approximately 5%–10% of HIV-positive who have more than 6 months HAART experience (Sulkowski, 2004; Wit *et al*., 2002; Reisler *et al*., 2001). According to the AIDS Clinical Trial Control Group grading, about 10.7% of the participants in this have been shown to have grade 1 elevation in serum AST. This is just a mild elevation in AST and may be associated with other causes other than hepatotoxicity. In most cases these elevations are seen in conditions such as non-alcoholic steatohepatitis (NASH), extrahepatic biliary atresia (EHBA), cirrhosis, fatty liver, myositis, drug toxicity, duchenne muscular dystrophy and even after vigorous exercise (Friedman *et al*., 2003; Daniel and Marshall, 1999). Several mechanisms associated with drug induced liver injury (DILI) have pathways that make use of the availability reactive species and the development of oxidative stress. Examples of these mechanisms include lipid peroxidation associated cell death (Negre-Salvayre *et al*., 2010; Kehrer, 1993) direct mitochondrial toxicity (Soriano *et al*., 2008) and cytochrome P450-mediated oxidative stress (Bansal *et al*., 2010; Koop, 2006). With the knowledge that

oxidative stress is associated with HIV infection and the use of ARV, its negative effects on the liver and other organs such as the kidneys and the brain are highly possible.

Elevations in serum ALT level is a more specific indicator of an ensuing liver disorder than AST. Alcoholism, smoking and other chemicals can cause injury to the liver, causing ALT levels to rise (Zabala *et al*., 2015; Tarantino *et al*., 2014). In this study however, there was reduction in ALT levels post-HAART. Only 9 (8.7%) of participants disclosed that they consume alcohol. None of the participants in the study used tobacco products, recreational drugs such as cocaine or consumed herbal medicines. A possible reason why ALT levels did not increase but rather decrease. As such it could be concluded in this study also that, ARVs did not cause an increase in serum ALT levels.

This study also sought to find an association between serum levels of vitamin E and zinc and liver function of the participants. This is based on the fact that, oxidative stress causes a depletion in these antioxidant micronutrients leading to their possible deficiencies. This low antioxidant status gives way to oxidative stress which causes deleterious effect on body organs such as the liver. Results from the study showed that there is no association between the serum levels of vitamin E and AST (p = 0.473) and ALT (p = 0.607) and serum levels of zinc on AST (p = 0.925) and ALT (p =0.470) (Table 4.9) and hence on, liver function.

The use of are also associated with the incidence liver disorders. In this study however, none of the participants was using these products as they were part of the exclusion criteria for participating in the study.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Dietary intake of antioxidant micronutrients among HIV positive people undergoing antiretroviral therapy at the Volta regional hospital in Ho revealed that there was low daily dietary intake of vitamins C, D and E at 54mg, 2µg and 3mg respectively. Vitamin A (828 µg) and zinc (8.47mg) intakes however were within recommended intake ranges.

Serum vitamin E deficiency was high (82.5%) among the participants possibly as a result of low dietary intakes. Levels of vitamin E in serum were lower in obese individuals compared to underweight people. Notwithstanding this, both groups indicated lower levels in relation to normal ranges. Intake of vitamin E did not however correlate positively with serum levels. Serum zinc levels were in the normal ranges for majority of the participants in the study. Dietary zinc intake however, did not have any association with serum levels in this study. The prevalence of underweight, overweight and obesity among the PLWH in the study were 11.70%, 21.40% and 11.70% respectively.

There was a significant increase in serum AST levels with a significant decrease in serum ALT levels after the initiation of HAART. Both median values however were within the normal range. A small proportion of participants (10.70%) had mild (Grade 1) elevation in serum AST levels. There was no association between the serum levels of vitamin E and zinc and serum AST and ALT levels. The findings of this study suggest that with good nutrition, PLWH can have an improvement in prognosis.

6.2 LIMITATIONS OF THE STUDY

- Only two antioxidant micronutrients were measured in the serum in this study. It would be best if more than two antioxidant micronutrients were measured. Financial constraint was the reason why more could not be measured in this study.
- It is generally difficult to infer cause and effect relationship from cross sectional data. The study design might have underestimated some of the real effects of dietary and other lifestyle habits and exposures were modified due to health concerns of the participants.
- All methods of dietary assessment have their limitation and associated criticisms. The two assessment tools used in this study, the 24-hour dietary recall and the food frequency questionnaire have weaknesses of depending entirely on a person's memory. This can introduce recall bias and may lead to underreporting or overreporting of type, amounts and frequencies of food intake.

6.3 RECOMMENDATIONS

Based on the information gathered from this study, the following recommendations are made;

- The Ghana Health Service and the Ghana AIDS Commission should help in restructuring nutrition related services to PLWH with emphasis on regular nutrition screening which will adopt a more holistic approach and include biochemical assessment so that micronutrients deficiencies can be determined and addressed in time. The issue of low vitamin E intakes and serum levels as

well as other antioxidant micronutrients should be broadly addressed due to its potential sub-clinical and clinical effects.

- Nutrition education and counselling in ART clinics must be intensified and the importance of consuming diet from variety of sources be emphasized.
- An increased frequency and consistency in the monitoring of AST and ALT among PLWHs should be considered and as such that more resources should be allocated to it.

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LIST OF APPENDICES

ANNEX 1: PARTICIPANT'S QUESTIONNAIRE

PART 1

Section A. PERSONAL INFORMATION

Please tick where appropriate.

Code…….. (*To be filled by researcher*) Date: ……/…...../20…..

1. Sex: A) Male B) Female

2. Date of birth…...../……../...…… 3. Age …...… (Years)

4. Level of Education: (please tick) A) Primary B) Junior high C) Senior high D) Tertiary E) None

5. Occupation: ……..……

6. Ethnic background: ……..

7. Place of residence: ……....

8. Income per month **A**) < GH¢ 100 **B**) GH¢ 100-300 **C**) GH¢400-600 **D**) GH¢ 700-900 **E**) GH¢ 1000-2000 **F**) GH¢ 3000-4000 **G**) Gh¢5000 and above

9. Marital status: A) Single B) Married C) Divorced D) Widowed

10. Contact number: ……..

Section B. MEDICAL HISTORY (from respondent)

11. Which year were you diagnosed of the disease? ……..

12. Have you missed any pills since you started ART? A) Yes B) No *If No, jump to* 14

13. If yes, how many pills have you missed? ……

14. Did you stop taking ARV drug for some time? A) Yes B) No *If No, jump to 17*

15. If yes, for how long? ……..

16. Why did you have to stop taking those drugs?

……

……

……

……

17. Do you have any adverse reactions to any of the drugs? A) Yes B)

No If *No jump to 19*

18. If yes for 17, please what are these reactions? (*Please list all*)

……

……

……

……

19. Have you taken any herbal medicine whiles on ART? A) Yes B) No

20. If yes, for how long? ……..

Section C: SOCIAL HISTORY

21. Do you use tobacco products? ……A) Yes B) No

22. How many years have you used tobacco? .....

23. Number of packs or sticks of cigarettes smoked in a day ……

24. Do you drink alcohol? …… A) Yes B) No *If No jump to 27*

25. If you drink alcohol, what do you drink? .....…….

26. How many tots/glasses per day? ……..……

27. If you use recreational (street) drugs, what do you use and how often? ……

Section D: ANTHROPOMETRY AND INVESTIGATIONS

Sample ID…….…… Collected by: ……

	Test	
28	Serum Zinc (µg/dl)	
29	Serum vitamin E (µmol/l)	
30	ALT (IU/L)	
31	AST (IU/L)	
32	GGT (IU/L)	

		1st reading	2nd reading	Average
33	Weight (kg)			
34	Height (cm)			
35	BMI (kg/m^2)			

Any other comments

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PART 2: SECONDARY DATA RESPONDENT'S MEDICAL RECORDS

Section E. MEDICAL HISTORY

No.	Infection/ Disease	Yes	No
36	Cancer		
37	Hepatitis B infection		
38	Cirrhosis		
39	Diabetes mellitus		
40	Hypertension		
41	Pulmonary Tuberculosis		

Section F. MEDICATIONS

i. Antiretroviral medications

No.	Antiretroviral drug	Please tick	Duration of intake (in months)
42	Abacavir (ABC)		
43	Emtricitabine (FTC)		
44	Ritonavir boosted Lopinavir (LPV/r)		
45	Stavudine (d4T)		
46	Didanosine (DDI)		
47	Nevirapine (NVP)		
48	Nelfinavir (NFV)		
49	Lamivudine (3TC)		
50	Efavirenz(EFV)		
51	Zidovudine (AZT/ZDV)		
52	Tenofovir (TDF)		

ii. Other medications

No.	Drug	Duration of intake (in months)
53		
54		
55		
56		
57		
58		
59		
60		
61		
62		
63		

Section G. please check patient's medical records and tick *yes* or *no* as applicable to respondent

No.	Symptom	Yes	No
64	Jaundice		
65	Abdominal pain and swelling		
66	Swelling in legs and ankles		
67	Itchy skin		
68	Dark urine colour		
69	Pale stool colour, or bloody or tar-coloured stool		
70	Chronic fatigue		
71	Nausea and vomiting		
72	Loss of appetite		
73	Tendency to bruise easily		

Section H. Pre - ARV Initiation Liver Function Test results

74	ALT (IU/L)	
75	AST (IU/L)	
76	GGT (IU/L)	

ANNEX 2: FOOD FREQUENCY QUESTIONNAIRE

Identification number	
DIRECTIONS: The questionnaire is to assess the number of times of fruits and vegetables, plant oils, fish oils food you have consumed over the **past 6 months.** Where possible provide **one answer to a question**	

Meal consumed	Code	Daily	Weekly (1-3 times)	Monthly	Occasionally	Never	Portion size
FRUITS							
Watermelon	F1						
Banana	F2						
Citrus (Orange, tangerine)	F3						
Grape fruit	F4						
Mango	F5						
Pineapple	F6						
Pawpaw	F7						
Apple	F8						
Pear	F9						
Guava	F10						
Others	F11						
VEGETABLES							
Tomatoes	V1						
Garden eggs	V2						
Kwansosaa(abedru)	V3						
Lettuce	V4						
Kontomire	V5						
Okra	V6						
Carrot	V7						
Cabbage	V8						
Other leafy dark vegetables	V9						
Ayoyo leaves	V10						
Dandelion leaves	B11						
STEW/ SOUP							
Tomatoes stew	S1						
Cabbage stew	S2						
Kontomire stew	S3						
Garden eggs stew	S4						

Light vegetable soup	S5						
NUTS AND OILS							
plant oils (Corn, soy, canola, coconut, sunflower oils)	N1						
Tiger nut	N2						
FISH OIL							
Salmon	E1						
Herrings	E2						
Anchovies	E3						
Tuna	E4						
Oysters	E5						
MEAT, POULTRY							
Lean beef	M1						
Lean Meat (cow, goat,	M2						
Lean chicken	M3						

NB: Kindly list any food you have consumed which did not appear on the food in the list above

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ANNEX 3: 24-HOUR RECALL QUESTIONNAIRE

Meal Time	Meal	Quantity (Handy measure)	Weight in gram
Breakfast Time…….			
Mid – morning Time ……			
Lunch Time …….			
Mid – afternoon Time……			
Supper Time……			
Bed time meal Time…….			

ANNEX 4: PARTICIPANT INFORMATION LEAFLET

Participant Information Leaflet and Consent Form

This leaflet must be given to all prospective participants to enable them know enough about the research before deciding to or not to participate

Title of Research: Antioxidant Micronutrients Intake in People Living With HIV: Implications on Serum Levels and Liver Function.

Name(s) and affiliation(s) of researcher(s): This study is being conducted by Daniel Edem Kpewou, Mrs F. O. Mensah and Mr Collins A. Appiah all of the Department of Biochemistry and Biotechnology of the KNUST, Kumasi.

Background (Please explain simply and briefly what the study is about): Owing to the oxidative stress associated with HIV infection and antiretroviral (ARV) therapy, it has become necessary to assess the intake of antioxidant based foods by people living with HIV (PLWHs) and their risk of developing liver disorders.

Purpose(s) of research: The main objective of this study was to assess the antioxidant micronutrient intake, serum levels and relationship with liver function among HIV positive individuals undergoing antiretroviral therapy at the outpatient department of the Volta Regional Hospital in Ho.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research: A total of 150 participants will be recruited into this research. A structured questionnaire would be used to assess respondents' demographic characteristics and medical histories. Anthropometric parameters i.e. height, weight, of the participants will also be measured. Five millilitres (5ml) of venous blood will be collected for analyses of specific liver enzymes, alanine aminotransferase (ALT), Aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and specific antioxidant micronutrients; vitamin E and Zinc.

Risk(s): There is no intended risk to participant in this study, however, a little discomfort and pain would be felt by participants during venepuncture to obtain 5ml of blood for the analyses.

Benefit(s): Participants will benefit by knowing their nutritional status and know what to eat through the nutritional counselling.

Confidentiality: All information collected in this study will be given code numbers. Names will be coded promptly and codes will be recorded to enable the research team trace when giving feedback to study subjects about their laboratory test results and

nutritional status. Abnormal result will be communicated to participants through the medical officer in charge of the ART clinic, who the research team is closely working with. The research team will adopt the highest form of confidentiality. No name or identifier will be used in any publication or reports from this study.

Voluntariness: Taking part in this study should be out of your own free will. You are not under any obligation to. Participation is entirely voluntary.

Alternatives to participation: If you choose not to participate, this will not affect your treatment in this hospital. You will not be discriminated against in any way should you decide not to partake in this study and the researchers will not begrudge you.
Withdrawal from the research: You may decide to withdraw from the research at anytime without having to explain yourself. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal: There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study. Please note however, that some of the information that may have been obtained from you without identifiers, before you chose to withdraw, may have been modified or used in analysis reports and publications. These cannot be removed anymore. We do however promise to comply with the terms to which you signed to earlier on.

Costs/Compensation: for their time and inconvenience, participants will be given snacks.

Contacts: If you have any questions and contributions concerning this study, please do not hesitate to contact **Mr Edem Kpewou** on **0501348740**.

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman
Committee on Human Research and Publication Ethics
Kumasi
Tel: 03220 63248 or 020 5453785

ANNEX 5: PARTICIPANT CONSENT FORM

CONSENT FORM

Statement of person obtaining informed consent:
I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:
I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME:_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to
_____ (Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (maintain if participant is non-literate): _____

ANNEX 6: KNUST/CHRPE ETHICAL APPROVAL LETTER

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

CHRPE/AP/170/17 — 13th March, 2017.

Mr. Daniel Edem Kpewou
Department of Biochemistry
and Biotechnology
KNUST- KUMASI.

Dear Sir,

LETTER OF APPROVAL

Protocol Title: *"Dietary Patterns, Serum Levels of Antioxidant Nutrients and the Risk to Developing Liver Damage among Out-Patient HIV Infected Individuals Undergoing Antiretroviral Therapy at the Volta Regional Hospital, Ho.*

Proposed Site: *Antiretroviral Therapy Clinic, Volta Regional Hospital, Ho, Ghana.*

Sponsor: *Principal Investigator.*

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A notification letter of 14th November, 2016 from the Volta Regional Hospital (study site) indicating approval for the conduct of the study in the Hospital.
- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Protocol.
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, beginning 13th March, 2017 to 12th March, 2018 renewable thereafter. The Committee may however, suspend or withdraw ethical approval at any time if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at the close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you Sir, for your application.

Yours faithfully,

Rev. Prof. John Appiah-Poku.
Honorary Secretary
FOR: CHAIRMAN

Room 7 Block J, School of Medical Sciences, KNUST, University Post Office, Kumasi, Ghana
Phone: +233 3220 63248 Mobile: +233 20 5453785 Email: chrpe.knust.kath@gmail.com / chrpe@knust.edu.gh

ANNEX 7: PERMISSION FROM THE VOLTA REGIONAL HOSPITAL

In case of reply the number and the date of this letter should be quoted
My Ref No. VRH/5/14
YourRef.No
Our GHS Core Values:

- People-Centred
- Professionalism
- Team Work
- Innovation/Excellence
- Discipline
- Integrity

GHANA HEALTH SERVICE
Your Health - Our Concern

VOLTA REGIONAL HOSPITAL
GHANA HEALTH SERVICE
P. O. BOX MA 374
HO. V/R.
E-MAIL: HVOLTA@YAHOO.COM
TEL: (03620) 27318-20/28207
Fax: (03620) 27323

14th November, 2016

THE HEAD OF DEPARTMENT.
DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY
KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
KUMASI

RE. LETTER OF INTRODUCTION

With reference to your letter dated **4th November, 2016** on the above subject matter, I write to inform you that, **Management** of the **Volta Regional Hospital- Ho**, has accepted your request to allow **Mr. Daniel Edem Kpewou** to use the facility for his research work on the topic

"Assessing the relationship between dietary patterns, serum levels of antioxidant nutrients and the risk to developing liver damage among out-patient HIV-infected individuals undergoing antiretroviral therapy at the Volta Regional Hospital"

He is to present a copy of his final research work to the Head of Administration of the Volta Regional Hospital-Ho.

Thanks

(DR. JOHN TAMPUORI)
MEDICAL DIRECTOR
VOLTA REGIONAL HOSPITAL, HO

Cc.
MR. DANIEL EDEM KPEWOU
KNUST
KUMASI