STUDY OF BIOAVAILABILITY OF TRACE ELEMENTS IN SELECTED INDIGENOUS FOODS AND THEIR POTENTIAL USE ON MANAGEMENT OF HIV AND AIDS

BY

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DECLARATION

I hereby declare that this is my original work that has not been presented for the award of a degree in any university.

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DEDICATION

I would like to dedicate this work to my father Tom Lusi and my late mother Teresa Lusi.

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First, I would like to thank Almighty God for the gift of life and good health during this project.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrometry
AI	Adequate Intake
AIDS	Acquired Immunodeficiency Syndrome
ARV	Anti-Retroviral Medicine
CD3+	Cluster Differentiation Factor 3
CD4+	Cluster Differentiation Factor 4
CD8+	Cluster Differentiation Factor 8
FAO/WHO	Food and Agricultural Organization of the United Nations/ World Health Organization
GOK-KHDS	Government of Kenya – Kenya Demographic Survey
GOK-BDDP	Government of Kenya-Busia District Development Plan
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
HAART	Highly Active Anti-Retroviral Therapy
HCT	Haematocrit
HIV	Human Immunodeficiency Virus
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
NASCOP	National Aids STI Control Program
NF_{kB}	Nuclear Factor Kappa B
ND	Not Detected
PLWHA	People Living With HIV and AIDS
RDA	Required Daily Allowance
RBC	Red Blood Cell

RDW	Red Blood Cell Distribution
RNA	Ribonucleic Acid

WORKING DEFINITION OF TERMS

Adequate intake	Amount of nutrient required based on approximation of the average nutrient intake by a population group or sub population that appears to be healthy.
Basal requirement	The amount of nutrients to prevent clinical signs of functional impairment.
Bioavailability algorithm	A mathematical model that attempts to predict bioavailability taking into account the form of the nutrients presence of dietary modifiers and nutrients status of individual where applicable.
Bioavailable nutrients	Nutrients in food that are absorbed and made available for use by the body
Dialyzable nutrient	Proportion of nutrient that can be present in the intestinal lumen and available for absorption
Incidence	The frequency of occurrence of any event or condition over a period of time and relation to the population in which it occurs
Iron deficiency	A state in which there is insufficient iron to maintain the normal physiological function of tissues such as the blood, brain, and muscles
Malnutrition	Any condition caused by deficient energy and nutrient intake, or by an imbalance of nutrients.
Normative requirement	The amount needed for prevention of functional impairment and for the generation and maintenance of reserve storage of the nutrients
Nuclear factor kappa B (NF_{KB})	A cellular transcription factor that is involved in replication of the human immunodeficiency virus.
Oxidative stress	The production of reactive oxygen species is greater than the available antioxidant defence.
Prevalence	The total number of cases of a disease in a population at a given time

ABSTRACT

HIV infection increases nutritional demands and vulnerability to malnutrition. Malnutrition worsens the effects of HIV infection by weakening further the body's immune system, resulting in earlier and faster progression to the end stage of AIDS. Food supplementation especially with essential elements improves nutritional and clinical status of PLWHA and may delay early use of ARVs. Although high levels of essential elements have been recorded in some indigenous foods, translating physiological requirements of PLWHA into amount of these foods that can deliver the RDA is still a challenge due to lack of information on the bioavailability of the elements in the foods. The purpose of this study was to determine bioavailability of the trace elements in selected indigenous foods and their potential use on the management of HIV and AIDS. This was a pre-intervention study on consenting PLWHA in Butula Division, Western Kenya. Pre-ART subjects aged 19 to 49 years in clinical stages 1 and 2 of HIV and AIDS were sequentially recruited from PLWHA visiting the local VCT centre. Socio-economic, clinical and nutritional status were determined using a questionnaire, whereas haematological and T- lymphocytes subsets were determined by flow cytometer. Levels of trace elements in serum: Fe, Zn, Cr and Se of the subjects and selected indigenous foods were estimated using atomic absorption spectroscopy. Indigenous foods found to contain high levels of the elements were used to make food supplement. Bioavailability of the trace elements in the food formulation was estimated using *in vitro* digestion procedure and results compared with algorithm estimates. Most PLWHA (77.3 %) had normal weight (Body Mass Index = 18.5 to 24.9) and 63.3 % had anaemia (Haemoglobin < 12 g/dL and Red Blood Cell Distribution Width < 15.2 %). Only few subjects (4.2 %) had low levels of serum zinc indicative of zinc deficiency (< 70 g/dL). Low levels of CD4+ cells was associated with low levels of Mean Corpuscular Haemoglobin Concentration ($r = 0.243$, $p > 0.05$) and high levels of serum zinc ($p = -0.195$, $p = 0.055$). Incidence of Herpes zooster was associated with high levels of Red Blood Cells ($r = 0.212$, $p = 0.017$) and Haemocrit ($r = 0.184$, $p = 0.039$). Whereas low serum zinc level was negatively associated with fever ($r = -0.195$, $p = 0.035$), headache ($r = -0.18$, $p = 0.017$) and loss of appetite ($r = -0.245$, $p = 0.007$). Starch, protein, animal and plants food sources accounted for 45.17 %, 21.9 %, 17.48 % and 73, % respectively of total foods consumed by PLWHA in the division. Mean contents of trace elements analysed in the foods were in the range of 10.7 to 6.8 mg 100g^{-1} for iron, 3.5 to 0.56 mg 100g^{-1} for zinc, 0.3 to 0.17 mg 100g^{-1} for chromium and 0.05 to 0.015 mg 100g^{-1} for selenium. Levels of bioavailable elements predicted by algorithms zinc (13.9 %) and iron (1.2 %), were generally lower than the levels of dialyzable zinc (34.4 %) and iron (24.2 %) obtained *in vitro* digestion procedure. On average indigenous foods in the study area had adequate levels of iron, selenium and chromium, which are moderately bioavailable but very low levels of zinc. Indigenous foods consumed in this division, if mixed in good proportion can provide RDA for trace elements that boost immunity of PLWHA.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Since the first cases were reported in USA in 1981 and Uganda in 1982, Acquired Immune deficiency Syndrome (AIDS) has caused death of more than 25 million people worldwide and currently over 33.4 million people are living with the disease (Willis, 2002; UNAIDS/WHO, 2009). In 2008 alone, about 2.0 million deaths were caused by AIDS and 70 % of the deaths were reported in sub-Saharan Africa. Furthermore, 67 % of the 33.4 million people currently living with the disease worldwide are in sub-Saharan Africa which is home to just over 10 % of the world population (UNAIDS/WHO, 2009). In Kenya, recent statistics reported a drop in prevalence rate from over 10 % in 1990s to 7.4 % in 2007 among adults aged 15 to 49 years (NASCOP, 2009). The AIDS has reduced average life span of Kenyans by 5 to 7 years and orphaned over 1.5 million children (GOK, 2004).

The AIDS has hit hardest those who are at their sexually active and productive years, thus causing loss of labour force both at national and household levels. In rural agricultural communities, morbidity and mortality have cut production of many crops by more than 40 % in the households affected (UNAIDS/WHO, 2001). In the health sector, medical services are overstretched due to increased occurrence of opportunistic diseases that have resulted in 45 % to 75 % of beds in public hospitals being occupied by AIDS patients (GOK, 2004). Transactional sex in Kenya; especially along the Northern corridor highway from Mombasa to Busia-Malaba continues to be one of the prime determinants of the HIV epidemic. Busia district is one of the hardest hit districts in Kenya with prevalence rate at 16 % among expectant mothers and 50 to 60 % of total admissions in the district hospital are due to HIV and AIDS related illness (NASCOP, 2005).

The HIV and AIDS is currently managed by use of a combination drug therapy; in Kenya the standard first line regimen for adult is a combination of staduvine, lamivudine and nevirapine or efavirenz, which are used simultaneously with prophylaxes (cotrimoxazole) for opportunistic infections. The drugs have clinical benefits of prolonging survival and reducing AIDS symptoms (NASCOP, 2005). Whereas Anti-Retroviral Therapy (ART) is increasingly becoming available and use of a combination drugs therapy is reducing AIDS symptoms, their efficacy is likely to be short lived due to cumulative drug resistance caused by high rate of HIV mutation during replication. The demanding regimen and side effects of the drugs are also forcing their users into strategic drug interruption, a condition that increases the rate of drug resistance (Stine, 2005). Furthermore, Anti-Retroviral (ARV) medicine sometimes alters nutrients metabolism, resulting in high blood cholesterol and triglycerides, liver and kidney damage, which require nutritional interventions (GOK, 2006).

Nutritional therapy has been embraced as an important part in care of people living with HIV and AIDS (PLWHA) as evidenced by dietary counselling offered at voluntary counselling and testing (VCT) and Prevention of Mother to Child Transmission (PMTCT) centres (NASCOP, 2005). The achievement and maintenance of optimal nutritional health among PLWHA optimizes existing immune system function, improves drug response and lowers medical care cost due to reduced susceptibility to opportunistic diseases (NASCOP, 2005).

The HIV infection causes chronic diarrhoea, anorexia, malabsorption, impaired nutrient storage, increased energy demands and altered metabolism that predispose PLWHA to malnutrition (Tang *et al*., 2005). Malnutrition worsens the effects of HIV infection by weakening further the body’s immune system. This synergistic interaction between

malnutrition and HIV infection results in increased vulnerability to infections, poor health, earlier and faster progression to the end stage of AIDS (GOK, 2006).

Deficiency of zinc, iron and selenium among PLWHA has been associated with faster clinical progression of HIV disease to AIDS, a condition that can be reduced by supplementation (Semba, 1999; Burbano *et al*., 2002; Baum *et al*., 1997). Iron deficiency has been linked with AIDS related death and 50% decline in CD4+ cell count (O' Bren *et al*., 2005). Chronic viral infection such as HIV depletes all antioxidants and overwhelms antioxidant viral defence mechanisms, resulting in build up of reactive oxygen metabolites that damage tissues and cells participating in innate immunity (Allard *et al*., 1998; Favier *et al*., 1994; McLemore *et al*., 1998; Chaturvedi *et al*., 2004). Supplementation of trace elements such as zinc, selenium and iron that act as co-factors of antioxidants may help to protect the body from damage by HIV-mediated oxidative stress.

Some studies have shown that supplementation of iron and zinc are associated with increased viral replication and enhanced bacterial infection (Tang *et al*., 1993; Tang *et al*., 1996; Bess *et al.,* 1992; Delanghe *et al.,* 1998; Brock 1993; Tanchou *et al*., 1988 and de Monye *et al.,* 1999). More recent studies carried out in developing countries where anaemia and zinc deficiency are endemic, associate supplementation of these elements with improved immune system (Mbakaya *et al.,* 2005; O' Bren *et al.,* 2005; Olsen *et al*., 2004). In Kenya, for example anaemia affects three out of four children under five years, one out of every two women of reproductive age and one out of every five men. About a half of Kenyan children below five years old are also at high risk of zinc deficiency (GOK, 1999).

1.2 Problem statement

Although high levels of the trace elements have been documented in some indigenous foods (Muchemi *et al*., 2007; Ogubi, 2008; Muriuki, 2009; World Food 2 Dietary Assessment System www.fao.org, 1996), the foods are rarely consumed locally, and are instead sold to buy refined foods such as white bread, which are poor in the nutrients (Muriuki, 2009). This may be due to either lack of knowledge and skills for preparing the indigenous foods or influence of modern lifestyle that encourages use of highly refined foods that are easy to prepare (Walingo, 2009). In cases where the indigenous foods rich in the elements are used, unfavourable meal composition due to high levels of inhibitors such as phytates, tannins, dietary fibres and polyphenols, reduces absorption of the essential nutrients (Hallberg and Hulthèn, 2000, Lönnerdal, 2000). High levels of the inhibitors may be reduced by traditional food treatment practices such as soaking, germination, milling, drying, fermentation, boiling, roasting and appropriate diet combinations (Walingo, 2009; Hotz and Gibson, 2007).

Appropriate combination of the indigenous foods could provide sufficient levels of the trace elements, but translating these foods into physiological requirements of PLWHA is still a challenge, due to lack of information on bioavailability of the trace elements in the foods. Furthermore, the RDA of trace elements used currently, are based on nutrient bioavailability estimated in a normal human gut and thus may not be appropriate for PLWHA. This is because, PLWHA often suffer from gastrointestinal disturbance caused by infections, which reduce absorption of nutrients (Silva, 1999). Although there are numerous causes of micronutrient deficiency among PLWHA, gastrointestinal disturbance, which is characterised by inflammation, vomiting and diarrhoea, may considerably influence absorption of trace elements from food (Silva, 1999). However, the effects of gastrointestinal disturbance can be

managed by intake of appropriate food combination, which also reduces absorption inhibitors and increases absorption enhancers, to raise levels of bioavailable nutrients.

Nutrients bioavailability in food can be estimated by *in vivo* or *in vitro* methods. *In vivo* methods use metabolic balance technique in animals or small scale human clinical trials, whereas *in vitro* methods use simulated gastrointestinal digestion of food and determination of nutrients solubility (Camara *et al*., 2005). Other methods of *in vitro* study involve nutrient dialysis through artificial membrane and estimation of nutrients uptake by caco-2-cells (Pèrez-Llamas *et al*., 1997; Beiseigel *et al*., 2007). Although *in vivo* methods determine bioavailability of nutrients in the natural physiological conditions where regulatory mechanism and transit time are not altered (Kayne and Lee, 1993), they suffer from large inter-individual variations and their use is limited due to high cost and ethical issues involved (Miller and Schricker, 1982).

On the other hand, *in vitro* methods are cheap, flexible and rapid (Miller and Schricker, 1982). Results of *in vitro* methods also correlate to nutrients bioavailability determined by *in vivo* methods, therefore providing a useful bioavailability index that allows ranking of foods (Weink *et al*., 1999; Au and Reddy, 2000). Some algorithms have been developed for quick assessment of bioavailability of a few nutrients (Hallberg and Hulthèn, 2000; Miller *et al*., 2007), but they generally have poor predictions. This because they do not factor degradation of inhibitors and enhancers resulting from storage and cooking, and the food composition data used with the algorithms are often incomplete and may not be accurate (Beiseigel *et al*., 2007; Yun *et al*., 2004). However, algorithms provide a cheaper and faster way of assessing bioavailability of nutrients from different diets, therefore are widely used in the dietary surveys. Algorithms are also used to screen foods for unknown dietary factors by comparing

their predictions with the more accurate *in vitro* or *in vivo* methods (Hallberg and Hulthĕn, 2000). Therefore, comparison of the elements bioavailability prediction from algorithms with the more accurate methods may provide information that could form a basis for improving their performance.

This study intended to determine bioavailability of selenium, zinc, iron and chromium in selected indigenous foods and their potential use in the management of HIV and AIDS.

1.3 Hypothesis

Indigenous foods do not contain sufficient levels of selenium, zinc, iron and chromium that are not bioavailable from foods when mixed in the right proportion, for use in the management of HIV and AIDS.

1.4 Objectives

1.4.1 General objective

To determine the nutritional, clinical, immunological and haematological status of PLWHA and determine levels of zinc, selenium, chromium and iron in indigenous foods and their bioavailability for use in the management of HIV and AIDS.

1.4.2 Specific objectives

i. To determine feeding habits and nutritional status of PLWHA in Butula division using a questionnaire.

ii. To determine body mass index (BMI), CD4+, CD3+, CD4+ and CD8+ cells counts and CD4+ /CD8+ ratio among PLWHA in Butula division.

iii. To determine serum levels of zinc among PLWHA in Butula division.

iv. To determine levels of zinc, iron, selenium and chromium in selected indigenous foods in Butula division.

v. To determine bioavailable zinc, iron, chromium and selenium in selected indigenous foods by use of *in vitro* digestion and algorithm model.

1.5 Importance and significance of the study

It is important to know levels of the trace elements in indigenous foods, feeding habits and nutritional status of the PLWHA. This is because, such information is used to select and mix foods in the right proportion that not only maximises levels and enhances bioavailability of trace elements but also is acceptable to the subjects. Information on bioavailability of the trace elements in indigenous foods is important because, it is used in translating physiological requirements of PLWHA into the amount of indigenous foods that can deliver required daily allowance (RDA) of the elements. Although bioavailability of trace elements in food is usually determined by intervention, the findings of this study indicate that algorithm and *in vitro* digestion procedure are also useful in assessing bioavailability of the elements from different foods.

In this study, local food materials were used to develop a food formulation that has a potential use on the management of HIV and AIDS. The study also profiled the trace elements status of the pre-HAART PLWHA at clinical stages I and II of HIV and AIDS, thus shedding more light on the interactions between the trace elements and HIV infection at asymptomatic stage. This report is therefore useful to VCT, PMTCT and other caregivers in addressing malnutrition problem among PLWHA.

1.6 Limitations and scope

Total levels and bioavailability of the trace elements reported in this study may have been affected by genetic factors, soil and weather conditions, use of fertilizers and state of the plants maturity at harvest. Since these conditions influence final levels of the trace elements in the grains and therefore, were not taken into consideration. The values reported in the study may not therefore, be generalizable to similar foods. Bioavailability studies were based on *in vitro* digestion and algorithm models that do not reflect the natural digestive system, especially the role of active transport and physiological condition of the gut of PLWHA that may influence the results. The findings of this study may not accurately reflect feeding habits and nutritional status of PLWHA, which depend on availability of food that changes with season but, baseline survey was done once.

This was a pre-intervention study, which determined the levels and bioavailability of trace elements in selected indigenous foods, and nutritional status and feeding habits of PLWHA in Butula Division. Findings of the bioavailability studies were used to prepare food formulation with high levels of trace elements, which if bioavailable could be used in the management of HIV and AIDS.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HIV infection and transmission

The HIV is transmitted through body fluids exchanged during sexual contact, contaminated hypodermic needle, contaminated blood or blood products and from mother to the foetus during birth or breast feeding (Willis, 2002). HIV infects cells by a process of membrane fusion that is mediated by its envelop glycoprotein (gp120-gp41, Env) and triggered by interaction of gp120 with CD4+ and co-receptor CXCKR-4 and CCKR-5 (Stine, 2005). The major target for HIV infection is cell expressing CD4+ molecule on their surface. However, some infections also occur in T-helper lymphocytes, mononuclear phagocytes, and dendritic cells in lymph nodes and microglia in brain (Fenyo, 1993). It may take up to 6 months for antibodies to HIV to be detectable in blood after infection with the virus.

2.2 Stages of HIV infection

The HIV infection can generally be classified into four distinct stages: primary infection, clinically asymptomatic, symptomatic HIV infection and progression of HIV to AIDS. The stages are briefly discussed in the following subsections.

2.2.1 Primary infection

At the early stage of infection, there is high rate of viral replication that leads to rise in viral load, which depletes CD4+ cells. The HIV depletes CD4+ cells by suppressing their DNA repairs mechanism and inducing other genes to initiate their own death. The virus also depletes CD4+ cells by filling CD4+ cell receptor, inducing CD4+ cells synctia formation and super antigen and auto-antigen formation (Stine, 2005). The depletion of CD4+ cells results in manifestation of HIV disease in form of Candida and other symptoms. However,

the immune system responds by producing HIV antibodies and CD8+ cytotoxic/suppressor cells that reduce the viral load in the patient (Mindel and Tenant-Flowers, 2001).

2.2.2 Clinically asymptomatic

Early infection stage is followed by latency stage, where the patient is completely a symptomatic. The high level of HIV antibodies and CD8+ cytotoxic cells reduce viral load and replication. The levels of CD4+ cells rise to normal limit of above 350 cells/mm^3 (Mindel and Tenant-Flowers, 2001). Although the levels of HIV in the peripheral blood drop to very low levels, the patients remain infectious and HIV antibodies are detected in the body.

2.2.3 Symptomatic HIV infection

After a long time, the immune system is severely damaged by HIV, due to over stimulation of immune system by the virus or high rate of HIV mutation in the body that results into varied and more pathogenic strains of the virus (Stine, 2005). In some cases, the body simply fails to keep up replacing the T-helper cells that are lost. As a result of failing immune system, more opportunistic infections and cancers emerge in almost all the body systems. Examples of the infections include Kaposis Sarcoma, Herpes simplex and tuberculosis (Stine, 2005).

2.2.4 Progression from HIV to AIDS

It makes the end stage of HIV infection, whereby the risk of death and opportunistic infections significantly increases and CD4 + T-cell count fall below 50,000/mL (Rizzardi and Pantaleo, 1999). The patient illness becomes more severe leading to death.

2.3 Diagnostic and confirmatory tests for HIV infection

The common laboratory screen for anti HIV-1 and HIV-2 antibodies is by means of enzyme-linked immunosorbent assay (ELISA) or rapid tests based on antigens that consist of viral lysates or recombinant of synthetic proteins that correspond to the immunodominant epitopes of the two subtype B HIV-1 variants and HIV-2 subtype A (Stine, 2005).

Rapid tests are based on serum filtration through a membrane coated with recombinant HIV-1 and HIV-2 antigens. Although HIV screening tests are faster and cheaper, they are usually less sensitive and therefore confirmatory tests are required (Brun-Vězinet and Simon, 1999).

The most widely used confirmatory test is Western Blot (WB). It involves nitrocellulose transfer of an HIV 1 or HIV 2 viral lysate after electrophoretic migration in poly acrylamide gel. It shows recognition of different constitutive viral protein by specific HIV 1 or anti HIV 2 antibodies. Although western block is a reference test, it is difficult to standardize and lacks sensitivity during the early phase of sero conversion. Other confirmatory tests include immunoblotting with synthetic protein and radio immunoprecipitation (Brun-Vězinet and Simon, 1999).

2.4 Management of HIV and AIDS

The recent drop in prevalence rate from over 10 % in 1990's to 7.4 % in 2007, among adults aged 15-49 years old was realized through prevention strategies such as awareness campaign in the media, voluntary testing and counselling at VCT and PMTCT centres (NASCOP, 2009). Other approaches used include participatory education and entertainment through drama and puppetry among youth and women groups, and distribution of free condoms. Blood transfusion that causes between 5 % to 10 % infections worldwide is controlled by

screening all blood donations. Stringent selection criteria for blood donors has also decreased prevalence rate among the donors from 7% in 1990's to 1.3% in 2004 (NASCOP, 2005).

Most opportunistic infections among PLWHA can be reduced by simply observing high hygiene standard in handling food and drinking water. Use of prophylactic drugs such as cotrimoxazole among immuno-suppressed patients reduces prevalence of pneumonia, malaria and diarrhoea by 60 % and related deaths 40 % (Stine, 2005; Wiktor *et al.,* 1999). In cases of severe immune suppression (CD4+ < 200 cells / mm^3) patients are put on ARVs to reduce AIDS symptoms (NASCOP, 2005).

The PLWHA also need psychosocial care to enable them deal with effects of stigma, blame, discrimination, constant fear of death and more importantly loss of ability to care for themselves and their family. The young HIV patients are forced to confront and plan for the reality of leaving their children motherless or fatherless when they die. These issues are currently addressed by Community Health Worker Volunteers (CHWV) operating under non-governmental organizations. They also link AIDS orphans and PLWHA with micro finance programmes to help them establish income-generating activities (NASCOP, 2005).

Nutritional management of HIV and AIDS is integral to medical care of all PLWHA, especially the ones at nutritional risks. Nutritional care of PLWHA is a process that involves nutrition assessment, nutrition diagnosis, nutrition intervention and nutrition monitoring and evaluation. Nutrition assessment aims at gathering information about current nutritional status and adequacy of the diet, and identifying risk factors for impending nutritional complications. Common methods used in nutrition assessment include: anthropometric and body composition assessment, clinical assessment, and dietary assessment. Nutrition

assessment helps in identifying an actual occurrence, or risk of developing a nutritional problem, thus enabling appropriate nutrition interventions in time. After nutrition intervention, a review and measurement of the individual's status at a scheduled follow-up time in order to provide an opportunity to adjust the nutrition care plan if the clinical status changes or other nutrition issues emerge (NASCOP, 2005). Good nutrition strengthens the body's ability to fight diseases and delays progression of HIV to AIDS (RCQHC, 2005a). Figure 2.1 shows the role of good nutrition in the management of HIV and AIDS.

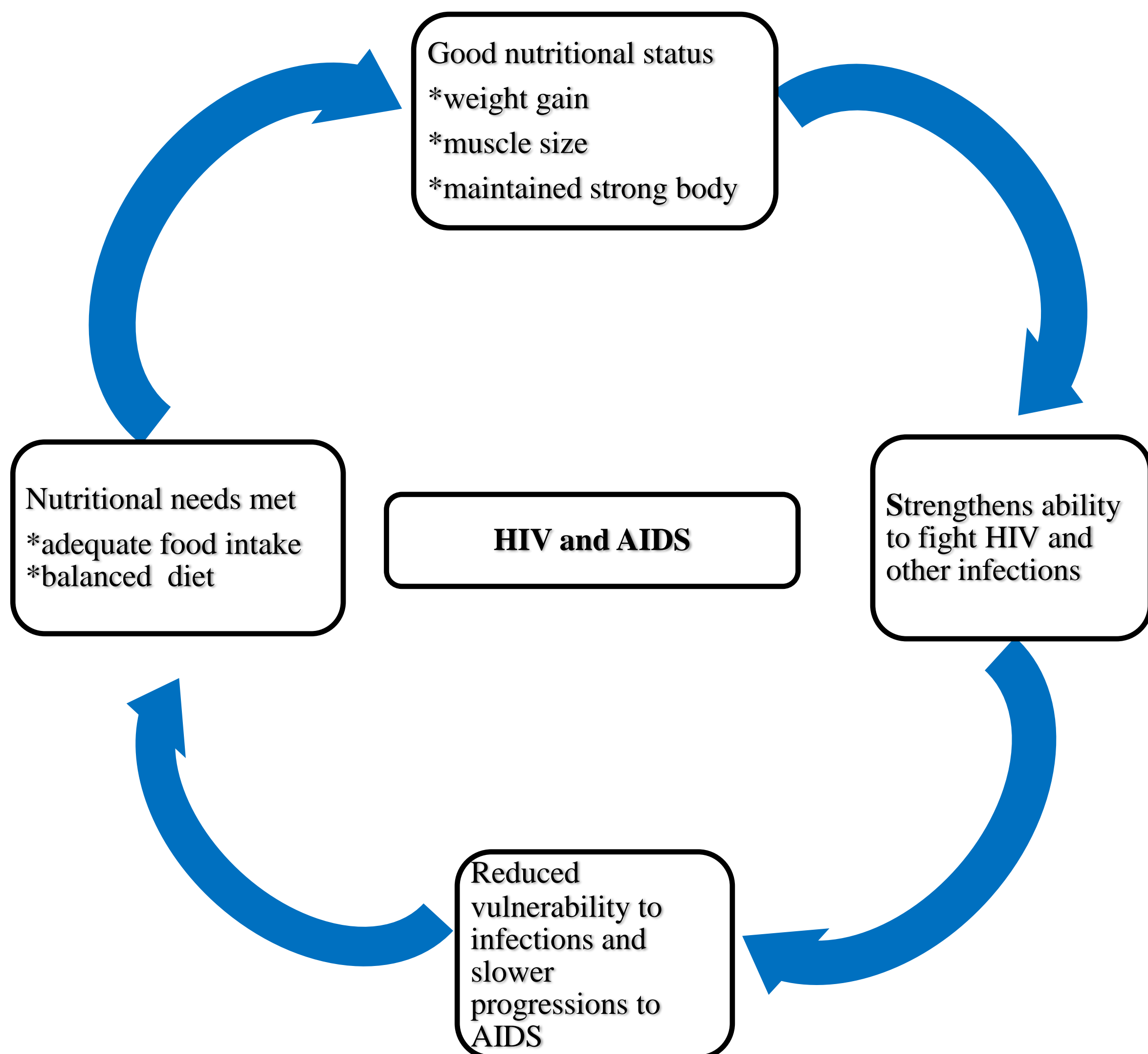

Figure 2.1 Importance of good nutrition

Source: RCQHC, 2005a

From the Figure 2.1, good nutrition meets the increased foods needs of PLWHA, especially energy and protein requirements that make them strong and maintain body weight. Good nutrition also increases levels of essential nutrients that strengthen their immune system enabling them to fight HIV and other infections, resulting in slower progression to AIDS.

Although there are two types of nutrition intervention: prophylactic micronutrients supplementation and therapeutic supplementation, only therapeutic supplementation is widely used and proven to be useful. Therapeutic nutrition is necessary during HIV and AIDS infection, to address complications that interfere with dietary intake of food and nutritional status, such as anorexia, constipation, lactose intolerance, diarrhoea, nausea and vomiting, early satiety, dysgeusia, gingivitis, mouth soreness, stomatitis and oesophagitis, xerostomia, dysphagia, fatigue and fever. Therapeutic nutrition is also used to reduce complications related to antiretroviral therapy, such as fat redistribution syndrome (lipodystrophy), dyslipidaemia, abnormal glucose tolerance and osteoporosis (GOK, 2006).

Nutritional status of PLWHA is also affected by drug-food interactions. Such interactions may involve effects of food on medication efficacy, effects of medication on nutrients utilization and effects of medication side effects on food consumption. The effects of such interactions can be reduced by therapeutic nutrition. Nutrition intervention is therefore, required at all stages of the disease, not only to delay progression, but also to manage side effects of ARVs and other medicine used to treat opportunistic diseases (GOK, 2006).

All nutrition interventions usually target replenishing immune boosting nutrients such as protein, vitamins, zinc, selenium, iron and chromium; this is due to high rate of cellular

turnover of immune system (Bogden and Olseke, 2007) and probably due to oxidative stress induced by HIV.

2.5 The immune boosting trace metals

Trace metals that boost the immune system include zinc, selenium, iron and chromium, these metals act as co- factors of enzymes that reduce oxidative stress generated by HIV and other infections.

2.5.1 Chromium

2.5.1.1 Chemistry and distribution

Chromium is a lustrous silver white metal, with a bluish tinge that belongs to group VI of the periodic table and has atomic weight of 52. The metal exists in any oxidation state from –2 to +6, but the common oxidation states are 0, +2, +3, and +6. In biological systems, however, only chromium (III) is stable as those above +3 oxidation state are oxidizing and those below are reducing (Mertz, 1969; Losi *et al*., 1994). Chromium is found in all the phases of environment, with soil having the highest levels of about 10 to150 mg/kg depending on the type of parent rock (Bertine and Golberg, 1971). In fresh water, chromium concentration is low and ranges from 0.1 mg L^{-1} to 6 mg L^{-1} (Bowen, 1979, Quoted in USEPA, 1989). Some of the most important ores of chromium include chromate ($FeCrO_4$), chrome ochre (Cr_2O_4), crocoisite ($PbCrO_4$) (De, 1992).

2.5.1.2 Dietary sources of chromium

Chromium level is generally low in staple foods ranging from 3.0 mg/kg in finger millet to 2.79 mg/kg in bulrush millet. Higher levels have been reported in herbal spices and seeds such as lemon grass (12.8 mg/kg) and ginger (10 mg/kg) (Muchemi *et al*., 2007). Processed

and canned acidic foods have been reported to contain high levels of chromium due to contamination from chromium in stainless steel containers and processors (Kumpulainen *et al.*, 1980). Uncontaminated drinking water is also known to contain extremely low levels of chromium < 1 µg/L. However, contaminated water due to industrial activities such as tanning leather, the steel industry and sites such as landfills, refuse tips and car scrap yards may have higher levels of chromium (EVM, 2002).

2.5.1.3 Absorption of chromium

Intestinal absorption of inorganic chromium (III) salt is low and is inversely related to dietary level. When low levels of 10 µg/day of chromium (III) is ingested, only 2 % is absorbed, whereas at high levels of 40 µg/day and above, only about 0.5 % is absorbed (Anderson and Kozlovsky, 1985). Hui (1992) attributed the low absorption rate of chromium (III) to high pH (above 4) prevailing in the intestine, that possibly causes hydrolysis and olation, leading to formation of inorganic molecules that cannot diffuse through the membrane.

On the other hand, absorption of inorganic chromium (VI) salt is relatively high in the intestine possibly due to high pH that allows existence of soluble forms of chromium (VI) ions that can diffuse easily across the membrane (Losi *et al.*, 1994). Absorption of chromium (VI) is thought to be controlled by reduction efficiency in the gastrointestinal tract, which varies by a factor of 3 or more depending on time of the meal (Frauchiger *et al.*, 2004). Organic complexes of chromium (III) and (VI) are also absorbed more readily, in the range of 10 to 25 % and 2 to 10 %, respectively (USEPA, 1985; Mertz *et al.*, 1978). Food components like vitamin C, picolinate, vitamin B and niacine form stable and soluble chromium complex at pH level in the intestine, thus enhancing absorption of chromium (Offenbacher, 1994). Other factors that promote chromium absorption include: increase in mucosal permeability,

inhibition of prostaglandin synthesis or presence of acidic luminal environment (Kamath *et al.*, 1997). A mechanism of chromium absorption has not been clearly elucidated, but it is thought to involve both active transport and passive diffusion (Basu and Donaldson, 2003).

Absorbed chromium is incorporated into glucose tolerance factor, a portion is bound to serum transferring and to other plasma proteins. Long term storage of chromium may be in the spleen, liver and bones (Lim *et al.*, 1983). However, a small portion of chromium circulates in blood in diffusible form, which is filtered and reabsorbed in the kidneys (Collins *et al.*, 1961), if excess is absorbed; some of it is excreted in urine (Offenbacher *et al*., 1984).

2.5.1.4 Chromium status

The reliable biomarker of dietary chromium and chromium status still remains a challenge to nutritional epidemiologist. Hair has been used in many studies as biomarker, but variability of chromium content of hair with level of chromium in hair washing agents such as shampoo is discouraging its use (Kumpulainen, 1982). Urinary excretion has been shown to reflect supplementary chromium in a dose dependent manner (Kumpulainen, 1983; Uusitupa *et al.*, 1983; Uusitupa *et al.*, 1992), but Anderson and Kozlovsky (1985) did not observe this relationship in dietary chromium. Magnitude of urinary excretion also depends on insulinogenic property of food (Anderson *et al.*, 1990). Increased lose of chromium is also observed during infection, lactation, pregnancy and acute exercise (Lukaski *et al*., 1997). The rise or fall in serum chromium level following a glucose load has been shown to correlate with chromium status (Liu and Morris, 1978; Liu and Abernathy, 1982). However, the use of serum or plasma remains a challenge, because normal chromium values are very close to the limit of detection of the analytical instruments commonly used, such as atomic absorption (AAS) and flame photometry (FP) (Veillon, 1989).

2.5.1.5 Immunological role of chromium

Chromium is an active ingredient of glucose tolerance factor that increases effectiveness of insulin and its ability to handle glucose, preventing hypoglycemia (too much insulin) or diabetes (too little insulin) (Dunne, 1990). Chromium supplementation has been shown to reduce cholesterol concentration, increase high-density lipoprotein (HDL) and decrease low-density lipoprotein (Mertz, 1982). Chromium level among PLWHA is low and decreases with use of ARVs (Aghdassi *et al.*, 2006). No report has directly linked chromium to complications among PLWHA, but it may help in reducing some complications such as elevated plasma triglycerides, elevated total cholesterol, decreased high-density lipoprotein concentration and type 2 diabetes.

2.5.2 Selenium

2.5.2.1 Selenium chemistry and distribution

Selenium is a semi metal that belongs to group VI b and period 4 of the periodic table. It exists in several allotropic forms which include ring chains of Se_8, amorphous (γ – selenium) and crystalline forms such as γ – monoclinic, β-monoclinic, and hexagonal (Johnson, 1976). The element is known to exist in several oxidation states that range from –2 in selenide to +6 in selenate, in over 20 compounds (CMBEEP, 1976). The major selenium ores include berzelianate, Cu_2Se; naumannite, $(Ag_2Pb)Se$; seebacthite, $2PbSe.4CuSe.Cu_2Se$ (De, 1992).

Most soils selenium soil content ranges from 0.1 ppm to 2.0 ppm (Mc Dowell, 1992), but higher levels have been reported in places where coal is used and copper sulphide is mined, and in arid places with sedimentary rocks (Foster and Sumar, 1995). Only small portion of selenium is available to vegetation. However, in well aerated alkaline soils, selenium salts are converted to selenite which is highly available to plants, thus such plants accumulate toxic

levels of selenium (McDowell, 1992). Other parameters affecting plants' uptake of selenium from soil include: levels of iron, phosphorus, sulphur, organic matter and clay in soil (Fordyce *et al.*, 2000). Use of selenium supplemented fertilizers also raises levels of selenium in soil which raises the levels in the plants (Ekholm *et al.*, 2007). Levels of selenium in water systems are low ranging from 0.1 to 100 µg/L (NRC, 1983), but higher levels are found in water systems of irrigated seleniferous soils.

2.5.2.2 Dietary sources of selenium

The major dietary sources of selenium are seafood products and offal or organ meat such as liver and kidney (Scherz and Kirchhoff, 2006). Moderate levels of selenium are found in cereals and grains that vary with amounts in soils where they are grown (FAO/WHO, 1998). A part from garlic and mushroom, vegetables generally contain lowest levels of selenium. In Kenya, levels of selenium range between 27.73 µg/kg in rice to 198.38 µg/kg in bulrush millet (Muchemi *et al.*, 2007). These levels show that with well balanced diet, Kenyan population may not be at risk of selenium deficiency.

2.5.2.3 Selenium status

Plasma selenium has been shown to respond rapidly to selenium supplementation and therefore, is regarded as a biomarker of short –term selenium status (Bùrk and Levander, 1999). However, at higher levels of intake, plasma selenium rise is not strongly correlated with dietary intake, but with the chemical form of selenium in the diet (Bùrk and Levander, 1999). Other biomarkers that have been used to assess selenium status include: whole blood, hair and urine. However, these parameters are prone to influence of other factors other than

selenium nutritional status. Plasma glutathione peroxidase is also useful in assessing dietary selenium intake, but only, when selenium status is in deficient range (Sunde *et al*., 1997).

2.5.2.4 Selenium absorption

Absorption of selenium occurs throughout gastrointestinal tract, with duodenum as a major site (Humaloja and Mykkänen, 1986). Selenium absorption is very efficient, normally in the range of 26.3% - 97% (Durcos *et al.,* 2005; Thompson *et al*., 1978), with higher retention of organic selenium compound than inorganic selenium compounds (Finely, 1999). In contrast to zinc and iron, selenium absorption is not homeostatistically regulated, but is under renal regulation (King, 2001). Very little is known about mechanism of selenium absorption, it may involve both carrier mediated process for organic compound and diffusion controlled process for inorganic selenium compound (Arduser *et al*., 1985: Raghib *et al*., 1986).

It is thought that, selenium that enters into the body goes into one of the three selenium metabolic pools in the body, depending on their levels and forms (Finley, 1999; Hawkes *et al*., 2003). In one pool, selenium is used to produce selenoprotein (temporary storage) and incorporated as seleno–cysteine into polypeptide chain (Bùrk and Hill, 1993). In another pool, certain selenium form is incorporated into metallothionen to form seleno-metallothionen, whereas all forms of the remaining selenium go into a third poll, where they are methylated and excreted through urine and lungs (Hawkes *et al.*, 2003).

2.5.2.5 Immunological role of selenium

Selenium maintains immunity system by acting as a co-factor of enzymes glutathione peroxidase and thioredoxin reductase that control concentrations of highly reactive oxygen containing metabolites in tissues. Selenium supplementation at 200 µg/day improves CD4+

cell response in HIV patients, acts as antiproliferative agent thus protecting against viral pathogens (Woods, 1999) and reduces the high levels of IL-8 and TNF-α associated with neurological damage, Kaposi's Sarcoma, wasting syndrome and increased viral load among PLWLHA (Baum, *et al.*, 2000). Other effects of supplementation include enhanced proliferation of active cytotoxic T cells and increased NK cell activity (Kiremidjian-Schumacher and Roy, 1998). Selenium deficiency enhances virulence of virus and impairs actions of neutrophils (FAO/WHO, 1998).

2.5.3 Iron

2.5.3.1 Iron chemistry and distribution

Iron is a transition metal that belongs to group VIII of the periodic table. It has four naturally occurring isotopes: iron – 56 (91.8 %), iron –54 (5.87 %), iron –57 (2.1 %) and iron –58 (0.3 %.). Iron exists in oxidation status ranging from 0 to +3; with oxidation +2 and +3 predominant in biological systems (CMBEEP, 1976). Iron (II) and iron (III) ions are often found in acidic aqueous and biological systems as hydrated ions $Fe(H_2O)_6^{+2}$ and $Fe(H_20)_6^{+}$, respectively, whereas in neutral and alkaline medium they lose protons to form corresponding hydroxides, $Fe(OH)_2$ and $Fe\ (OH)_3$ (Spiro and Saltman, 1974; Crichton, 1991).

2.5.3.2 Iron absorption

Absorption of iron takes place throughout the gastrointestinal tract, with the major sites being the duodenum and the upper jejunum (Crichton, 1991). In the acidic medium of the stomach, non-heme iron occurring as tight bound organic iron (III) molecules in foods is solubilised, ionized and reduced into iron (II) ions (Basu and Donaldson, 2003). The free iron (II) ions complex with other compounds in food such as amino acids, fructose, and ascorbic acids

which enhance its absorption into intracellular iron pool in mucosal cells (Williams, 1985). The above factors are thought to enhance iron absorption by formation of complexes that remain stable and soluble at the alkaline pH of the gastrointestinal tract (Crichton, 1991). In addition to the above role, amino acids are thought to buffer pH of the gut thus delaying increase of the pH towards neutrality where iron is oxidized into less soluble ferric ion, whereas ascorbic acid reduces ferric iron in food into a more soluble ferrous iron (Kies and McEndree, 1982; Crichton, 1991).

Heme iron is absorbed intact as metalloprophyrin complex by receptor mediated process involving specific high affinity mucosal brush border heme binding sites (Crichton, 1991). The absorbed heme iron is degraded by heme oxygenase and the free iron enters the common intracellular iron pool, where it combines with a protein receptor, aproferritin to form ferritin (Crichton, 1991; Williams, 1985). Upon entering the blood plasma, iron (II) from ferritin is quickly oxidized to iron (III), which is immediately bound with β-globin transferrin for transport to bone marrow for hemoglobin synthesis and to cells for iron containing cells (McDowell, 1992).

Excess absorbed iron is transported to liver and bone marrow, where iron is transferred to apoferritin as a stable storage form. Hemosiderin is another storage form that is less soluble and only present in iron overload (Williams, 1985). It is thought that the amount of iron entering the body is controlled by quantity of ferritin and transferrin present at the mucosal cells and the unbound iron remaining in lumen is discarded into gastrointestinal tract when the cells slough off (Chrichton, 1991; Hunt and Roughhead, 1999).

2.5.3.3 Iron status

Wide variations of bioavailability of iron and physiological processes make it impossible to use one reliable biomarker of the body iron status (Hambridge, 2003). The most sensitive indicator of iron store is plasma or serum ferritin, whereby 1 mg ferritin/L plasma is proportional to approximately 8 mg of stored iron in adult (Bothwell *et al.*, 1979). However, high plasma ferritin has been associated with neoplasm (Nelson *et al.*, 1994), infection, ethanol consumption (Legget *et a*l., 1990) and hyperglycemia (Tuomainen *et al.*, 1999). Hunt and Roughead (1999) did not observe any association between 6-fold difference in dietary iron bioavailability and serum ferritin or fecal ferritin.

Whereas haemoglobin and hematocrit measurements are simple to carry out and their low values are clear indication of iron deficiency, they are not specific and sensitive enough for mild iron deficiency (Hambidge, 2003). Although serum level, generally, poorly reflects the pool and lacks specificity, it is widely used in clinical practice epidemiologic research because, it is easy to determine and is practical. Other biomarkers of iron status include plasma soluble serum trasferrin receptor concentration, serum transferrin saturation, erythrocyte protoporphyrin concentration and red blood cell indices. Generally, the precision of the laboratory diagnosis or iron deficiency anaemia can be improved by combining hemoglobin with other indices of iron status (ESWG, 1985).

2.5.3.4 Immunological role of iron

Iron is a co-factor of many enzymes involved in immune functions such as catalase and cytochromes. Catalase is an antioxidant that removes reactive oxygen radical which enhances viral replication by activating nuclear transcription factors such as NF-κB (Staal *et al.,* 1993; FAO/WHO, 1998). Iron deficiency reduces IL-1 production, decreases oxidation burst of

neutrophils and lowers concentration of iron-based myeloperoxidase (Chester and Arthur*.,* 1988). There is a need for balanced intake of iron as high intake may lead to increased oxidative stress and viral replication (Delanghe *et al.,* 1998).

2.5.4 Zinc

2.5.4.1 Zinc chemistry and distribution

Zinc is a bluish white divalent metal, with atomic number and mass number of 30 and 65.37, respectively (Mc Dowell, 1992). Zinc ion has higher affinity for electrons, due to high charge of +2 concentrated in a small radius of 0.65A; these properties make zinc enzymes attack more rapid. In normal soils, zinc content ranges from 10 to 300 ppm, but soils near highways have higher levels due to contamination from zinc oxide and zinc dithiophosphite additives in vehicle tires and motor oil, respectively (NRC, 1979).

2.5.4.2 Dietary sources of zinc

The major dietary sources of zinc include lean red meat, whole grain cereals, pulses and legumes, whereas fish, roots and tubers provide only modest amounts of zinc (Dunne, 1990). Levels of zinc in grains reported in Kenya are in the range of 17.89 mg/kg in sorghum (Muchemi *et al*., 2007) to 28.78 mg/kg in Bulrush millet, which are comparable to levels reported in India, that range between 10.8 mg/kg to 25.7 mg/kg (Hemalanthan *et al*, 2007). Although grains and cereals in Kenya contain adequate levels of zinc, high levels of anti-nutrients such as phytate and polyphenols may reduce its bioavailability significantly.

2.5.4.3 Absorption of zinc

Absorption of zinc is thought to involve both saturable carrier mediated components and non –specific unsaturated diffusion controlled process (Steel and Cousins, 1985). When food is taken, the complex zinc containing species is broken down by digestive process into free zinc (Cousins, 1982), which is then complexed by ligands from pancreas, food and other endogenous factors such as citric acid, picolinic acid, histidine and cysteine (Basu and Donaldson, 2003). The complexed zinc metal is then transported through the micro villus into hypothetical 'zinc pool' where it is picked by albumin and ferritin for onward transfer to liver (Evans, 1974; Cousins, 1982). In liver, zinc is stored, processed and bound to metallothionen for redistribution (Cousins, 1982).

It is thought that the amount of zinc that enters the body is regulated by quantity of metal – free albumin available at the basolateral membrane, whereas serum zinc level is controlled by metallothionen (Evans 1974; McMahn and Cousins, 1998). A transmembrane polypeptide and zinc transfer proteins found in the crypts and lower villi are also known to control absorption of zinc (McMahn and Cousins, 1998). The remaining zinc in the intestinal cells is sloughed off along with some cells and excreted, thus preventing over absorption of zinc (Krebs, 2000).

2.5.4.4 Zinc status

Small decrease in tissue zinc that are associated with zinc-deficient morbidity, effectiveness of homeostatic mechanisms in maintaining plasma or serum zinc concentration and dependence on zinc of many aspects of physiological processes, have largely contributed to limitations of the currently used zinc biomarkers (Jackson *et al.,* 1982; Chester, 1978).

In spite of the limitations, plasma zinc is currently the most widely used and accepted zinc biomarker. Some studies have reported no association between zinc intake and plasma zinc (Thomas *et al.*, 1988; Neggers *et a*l., 1997), but association has been observed between intake of bioavailable zinc and plasma zinc (Donovan and Gibson, 1985). Other biomarkers of zinc status include cellular components of blood, hair zinc, zinc excretion, activity of zinc dependent enzymes, and measurement of zinc exchangeable pool sizes by stable isotope tracer techniques and metallothionein mRNA assays. The recent advancement in isotope techniques in nutrition research has made the later a method of choice for evaluating the relationship between diet and zinc status and a possible insight of homeostatic regulations of zinc (Hambidge, 2003).

2.5.4.5 Immunological role of zinc

Zinc is a co-factor of copper-zinc dismutase, which plays a very important role in protecting cells participating in innate immunity from damage by reactive oxygen radical (Erickson, *et al.*, 2000; Chaturvedi, *et al.,* 2004). Zinc ion inhibits intracellular HIV replication, because HIV replicates preferentially in Th-0 and Th-2 cells, but not in Th-1 cells which contain more zinc (Spreitsma, 1997). Furthermore, the zinc-bound form of thymulin (active thymulin) is reduced in stage IV of HIV and AIDS with decrease in CD4+ cell counts and increase in unbound form of thymulin (inactive thymulin). This condition reverses in vitro addition of zinc to plasma, indicating low zinc bio-availability as the cause of impaired thymic functions with consequent depletion of CD4+ cells (Mocchegieni and Muzzioli, 2000). Zinc deficiency reduces CD4:CD8 ratio, helper T-cells functions and promotes Th-2 response at the expense of Th-1. The Th-2 provides humoral immunity that is ineffective in fighting viral infections and enhances the ability of HIV virus to infect other cells (Prasad, 2007).

2.6 Influence of dietary factors on bioavailability of selenium, zinc, iron and chromium

Although most Kenyan indigenous cereals have high levels of the trace elements (Muchemi *et al*., 2007; Muriuki, 2009), bioavailability of the elements in these foods may be very low, due to poor dietary habits, unsuitable cooking methods and presence of high levels of inhibitors such as phytates, tannins, polyphenols and dietary fibres in the foods (WorldFood2 Dietary Assessment System www.fao.org, 1996; Walingo, 2009).

2.6.1 Phytate

Most important phytate inhibitor is phytic acid (myo inositol hexaphosphate), that reduces iron absorption by about 59 % (Sandberg *et al*., 1999). Phytic acid acts as the main storage form of phosphorus in cereals, legumes and oil seeds (Lopez *et al*., 2002). It has 12 replaceable portions and high density of negatively charged groups (6 phosphates) that allows it to form insoluble chelates with multivalent cations, such as Ca^{2+}, Zn^{2+}, Cr^{3+}, and Fe^{3+} (Perez *et al*., 2001). Lesser inhibitory effect has also been observed with myo-inositol pentaphosphate, whereas the remaining smaller phosphate groups do not have inhibitory effect, but can interact with higher phosphate groups, resulting in enhanced binding capacity (Sandberg *et al*., 1999).

Phytate is known to inhibit both zinc and iron in a dose dependent manner (Fredlund *et al.*, 2006; Hurrell *et al.,* 1992), with 15:1 ration of phytate to zinc predisposing one to zinc deficiency (Ferguson, *et al*., 1989). High levels of calcium enhance inhibitory effects of phytate and when (calcium X phytate): (zinc) millimolar ratio exceeds 200; zinc bioavailability is significantly reduced (Ellis *et al*., 1987).

Although there is very little information on inhibitory effect of phytate on selenium and chromium absorption, their absorption may also be reduced by complexation with phytate because they are multivalent cations just like zinc and iron.

2.6.2 Polyphenols

Polyphenols are secondary metabolites of plants that are known to protect plants against harmful effects of ultra violet radiation from the sun or aggression by pathogens. They are known to contain several hydroxyl groups and aromatic rings, which bind multivalent cations such as iron, zinc, selenium and chromium (Hurrel *et al.*, 1992). Most potent polyphenol inhibitors are chlorogenic acid, gallic acid, catechol, resorcinol, flavanols and ferulic acid, which belong to phenolic group. Generally, polyphenols are found in coloured foods, such as sorghum, millet, tea, coffee, onions, kales and broccoli (Matuschek and Svanberg, 2005).

2.6.3 Dietary habits

Taking tea, coffee or cocoa with or immediately after meal reduces bioavailability of trace elements, because they contain high levels of chlorogenic acid and tannins that form insoluble complexes with elements. It is estimated that a 150 mL cup of coffee and a 200 mL cup of tea reduce non-heme iron absorption by about 60 % and 75 to 80 %, respectively (Hallberg and Hulthèn, 2000).

Milk tea or milk coffee with sugar contains fructose and protein that could enhance absorption of trace elements, such effects are controversial for casein (major protein) in milk (Sandstrôm *et al.*, 1983), and can only be realized at higher concentration for fructose, at ratio of 100:1 of fructose to iron (Pollycore *et al.*, 1972). Whereas Rosado *et al.* (2005) reported

that taking 250 ml of milk with plant based meal increases fractional zinc absorption by 50 %, no such an effect was observed by Galan *et al.* (1991) and another study reported negative effect on zinc absorption (Sandstrôm *et al.,* 1983). Furthermore, a number of studies have consistently reported no effect of milk on iron absorption (Deer *et al*., 1990; Rosado *et al*., 2005). Therefore, milk in tea or coffee has little effect on bioavailability of the trace elements.

It is postulated that in cow's milk, zinc and iron are bound to phosphoserine groups in casein micelles (Hekmat and McMahon, 1998; Singh *et al.*, 1989) and colloidal calcium phosphate especially for zinc (Singh *et al*., 1989). These compounds bind the elements strongly in form of aggregate macromolecule that are too large to be absorbed (Rosado *et al.*, 2005). Furthermore, high levels of calcium in milk can interact with phytate in plant based meals to reduce bioavailable zinc, by formation of insoluble calcium – zinc-phytate complex (Ellis *et a*l., 1987). However, the macromolecule can be broken down by acidification and fermentation of milk, thereby causing hydrolysis of milk protein and solubilization of bound elements (Drago and Valencia, 2002).

Consumption of large amount of food in a sitting may contribute to intake of large amount of fibre which reduces bioavailability of trace elements. It is thought that increase in weight and bulk of undigested residues in gut, decrease food transit time, especially the contact time between villi and the elements, thus reducing opportunity for absorption (Reinhold, 1982). Dietary fibre, due to its abrasiveness sloughs off mucosal epithelium which acts as excretion pathway for some trace elements such as iron and zinc, thus preventing reabsorption of the elements. Other side effects of high dietary fibre include formation of insoluble complexes

with trace elements as pH approach 7.0 and conversion of soluble iron (II) to insoluble iron (III) (Reinhold, 1982).

In contrast, binding of trace elements such as iron by dietary fibre is strongly inhibited *in vitro* by ascorbic acid, citrate, cysteine or phytate, unfortunately all these compounds have poor uptake of fibre bound elements (Reinhold, 1982).

2.6.4 Type of protein

Absorption of trace elements from legume protein sources is lower than from animal protein sources. This is mainly attributed to inhibitory effects of phytic acids found in plants. Other factors that influence absorption of trace elements from protein include the composition of amino acids and the existence form of the element. Hurrel (2003) suggested that low bioavailability of iron in soy protein is due to presence of glycinin and conglycinin forms of protein that inhibits its absorption. The effect of composition of amino acids was also shown by Perez-Llamas *et al.*, (1997) in their study of the effects of pork, beef and soy protein on dialyzability of iron and zinc, where the highest dialyzable iron was observed in animal proteins, while the lowest dialyzable iron was observed in soy protein.

High rate of iron absorption from animal protein is due to presence of highly bioavailable iron in the heme form, which does not exist in plant protein sources (Hunt and Roughead, 1999). However, some animal protein sources such as milk and eggs contain trace elements that have low bioavailability. Substitution of eggs for other proteins reduces iron absorption significantly, with one egg corresponding to a reduction of 27 % (Hallberg and Hulthĕn, 2000). This may be due to presence of phosvitin that inhibits the absorption of non-heme iron in adults (Cook and Monsen, 1976). Casein phosphate and whey protein in milk inhibit

absorption of trace elements (Jackson, 1992). Direct interaction between trace elements and high levels of calcium in milk also inhibits absorption of the elements in milk (Ekmekcioglu, 2000).

2.6.5 Food preparation and storage

2.6.5.1 Effect of heat

Whereas bioavailability of trace elements is enhanced by cooking, because heat softens the food matrix and release protein bound trace elements (Lombardi Boccia *et al*., 1995), over boiling destroys ascorbic acid by oxidation at higher temperatures, thus reducing bioavailability of the elements (Clydesdale, 1982). Ascorbic acid enhances absorption of trace elements by forming soluble complexes with the elements and it reduces insoluble ferric iron to soluble ferrous iron in food (Crichton, 1991). Therefore, absorption of trace elements can be enhanced by taking meals with a glass of fruit juice that is rich in ascorbic acid such as citrus fruits.

2.6.5.2 Mechanical processing

Dehulling and household pounding remove the bran and germ from cereals, this reduces phytate content, especially when it is localized in the outer a leurone layer or in the germ (Kebede and Urga, 1995; O'Dell *et al.*, 1972). Reduction in phytate content in cereals increases bioavailability of the trace elements in cereals.

2.6.5.3 Germination

The most effective way of reducing phytate content in cereals is by germination. Germination activates endogenous phytase enzymes, which convert phytic acid into lower inositol phosphates that have poor capacity to bind minerals (Sandberg *et al*., 1999). Germination reduces phytate content in cereals by 53%, 67% and 27% in red sorghum, millet and maize after 74, 62 and 66 hours, respectively (Traore *et al*., 2004). In cereals with low levels of phytase enzymes such as rice and maize, levels of phytate can be reduced by soaking the cereals (Egli *et al*., 2002). Perlas and Gibson (2005) reported that soaking maize for six hours, followed by decanting the soaking water prior to preparing porridge, reduces penta and hexa inositol phosphate by 18 to 42%. They attributed reduction to diffusion of water soluble sodium, potassium or magnesium phytate in maize which are thought to constitute 70 % phytate present in maize. However, soaking of flour and whole grains of some cereals also causes loss of minerals through diffusion, but the losses are balanced by phytate degradation (Lestienne *et al*., 2005).

2.6.5.4 Fermentation

Several studies have shown that, fermentation improves bioavailability of trace elements by reducing levels of antinutrients, such as phytate and tannin (Onyango *et al*., 2004; Kebede and Urga, 1995). Organic acids produced during fermentation, such as citric, malic and lactic acid form soluble ligands with trace elements and lower pH that optimizes activity of endogenous phytate from cereals or legume flours (Chang, 1967; Teucher *et al*., 2004).

Use of iron cooking ware for preparation of acidic foods may solubilize iron, thus increasing its availability in food. This is thought to be the cause of high levels of iron among consumers of traditional beers in South Africa (Green *et al*., 1968; Emery, 1991). It is also thought that

high levels of chromium in soft drinks of tea, lemon and orange juice is due to its contamination from steel processing plants (Garcia *et al*., 1999).

2.7 Determination of bioavailability of elements in food

Recent technological advancements have improved accuracy and precision of methods used in study of bioavailability and absorption of trace elements. However, in comparison to other fields of study, analytical procedure used in study of bioavailability and absorption of trace elements are still low in precision and accuracy. Currently two models are used to study bioavailability of elements from foods, and they include: *in vitro* and *in vivo* models.

One of the *in vitro* models commonly used involves spectroscopic determination of ionizable trace elements in food. This approach involves subjecting food to a simulated gastrointestinal digestion by adjusting pH according to physiological range and use of purified peptic acid, solution of physiological amount of elements, pancretin and bile solutions (Matuschek and Svanberg, 2005). A variant of the method involves determination of dialyzable nutrient through artificial membrane (Pèrez-Llamus *et al*., 1997) or nutrient uptake and transport by caco-2-cells (Beiseigel *et al*., 2007).

In *vitro* ligated intestinal loop procedure involves introducing radio tagged food into ligated intestinal loop of anaesthetized animal, at end of the absorption, the animal is slaughtered and the unabsorbed element in the loop is determined (Humaloja and Mykkänen, 1986). This method enables study of absorption of the labelled elements from different gastrointestinal segments. However, it does not factor the effect of food transit in the gut. The recently developed *in vitro* method uses cultured human intestinal cells to estimate uptake of nutrients from food. The model is rapid, cheap, and flexible and enables ranking of selected foods on a

chosen food standard, but it does not closely resemble human gastrointestinal system (Campen and Glahn, 1999).

Ratio of the trace element to inhibitors is also used to predict bioavailability of trace elements in food (Fredlund *et al*., 2006; Murphy *et al*., 1992). Although the *in vitro* bioavailability studies are cheap, convenient, faster, precise, and flexible and may be sensitive to type of food and food treatment, they do not reflect the natural digestive system, especially the role of active transport and brush border binding proteins (Miller and Schricker, 1982).

In vivo models commonly used to study bioavailability and absorption of trace elements include: animals such as pigs, rats, dogs and poultry and small scale human clinical trials. Use of such animals' models offers advantages of close resemblance to human because intact gastrointestinal system is used, less expensive and flexible, but are associated with errors of extrapolation to humans. Small scale human clinical trials appears to be the best method that is free from extrapolation errors associated with other models, however, its use is limited due to ethical issues and high costs involved. The method also suffers from low precision due to influence of factors that are not related to food such as nutrient status, appetite and integrity of gastrointestinal tract (Miller and Schricker, 1982). The commonly used methods *in vivo* studies include metabolic balance and everted gut sac technique. Metabolic balance study is currently considered to be the best technique for determining absorption and bioavailability of trace elements in foods. However, it does not differentiate absorption from other metabolic processes and gives no information about luminal interactions (Humaloja and Mykkänen, 1986). In metabolic balance technique, bioavailability of trace elements may be estimated by use of isotope tracer technique or rate of serum repletion of the trace elements.

2.7.1 Isotope tracer techniques

In these techniques, bioavailability of trace elements in food is directly determined by tracing the fate of intrinsic or extrinsic labelled element in consumed food (Weaver, 1984). Isotope tracer techniques can be classified into two major groups: radio isotope and stable isotope technique.

2.7.1.1 Radio isotope tracer technique

In this technique, intrinsic or extrinsic radio isotope labelled food is given to the study subject and retention of the radio isotope in the whole body or in the blood is measured overtime (Hunt and Roughead 1999; Bothwell *et al*., 1979). This method has been widely used in bioavailability and absorption studies, because it is relatively cheap and uses very accessible technologies such as scintillation counting (Christie *et al.*, 1984). In addition, it is simple since no frequent food sampling is required and intact biological system is used (Miller and Schricker, 1982). However, use of this method is currently discouraged among humans, due to risk of radiation exposure, especially in expecting women and infants.

2.7.1.2 Stable isotope trace technique

This technique is similar to radio isotope technique, with exception that, stable isotope is used in labelling food. Bioavailability of the elements in food is estimated by determining levels of other retained tracer isotope in faeces and urine or absorbed trace isotope in blood (Shaw *et al*., 1984). Stable tracer isotopes are quantified by use of mass spectrometry or neutron activation analysis method, based on isotope dilution principle. Unlike radio isotope technique, there is no radiation risks in this technique, and it is considered as the most accurate, since quantitative recovery of analyte is not required and the enriched spike isotope

serves as an ideal internal standard. However, use of this method is not as wide spread as radio isotope technique because analytical separation procedures involved are time consuming and costs of instrumentation used is prohibitive for most laboratories (Veillon,1984).

2.7.2 Intrinsic labelling

In this technique, the tracer element is incorporated into the food during the normal biological growth or process; it is also referred to as biosynthesis of food using the tracer elements. For example, food grains such as green grams may be germinated, and then transferred to a circulating hydroponic nutrient solution, where a tracer element is introduced at the flowering stage of the plant (Weaver, 1984). In study of absorption of elements from animals based food sources, the animal may be labelled by the intravenous injection of the tracer or fed food labelled with a tracer (Sirichakwal *et al.*, 1985; Campen and Glahn, 1999).

Although some studies have shown that there is no difference between absorption of intrinsic label and extrinsic label (Sirichakwal *et al*., 1985; Mason and Weaver, 1986), other studies have reported that extrinsic isotope label is better absorbed than intrinsic isotope label (Thomson *et al*., 1975; Richold *et al*., 1977). It is thought that intrinsically labelled food sources better represent absorption of the elements than extrinsically labelled; this is because isotope in food matrix may not readily exchange with added extrinsic labels. Perez- Llamas *et al.* (1997) also reported that absorption of intrinsic labelled zinc is over three times higher than that of extrinsic labelled zinc. This is due to lack of influence of absorption mechanism that is largely a carrier mediated process. Generally, most studies use extrinsic labelling technique, because intrinsic labelling technique is time consuming and cumbersome (Weaver, 1984).

2.7.3 Extrinsic labelling technique

Extrinsic labelling is carried out by adding aqueous solution of tracer isotope to the study food, and allowed to exchange with endogenous isotope elements in the food (Weaver, 1984). The bioavailability may be estimated by *in vitro* methods such as dialyzability of the element or *in vivo* methods such as estimation of rate of absorption by monitoring levels of the tracer isotope in faeces and / or urine. Extrinsic labelling technique is commonly used in absorption and bioavailability studies, because it is simple to carryout and less time consuming compared to intrinsic labelling method. However, there is a major concern with this technique that the added isotope traces may not exchange completely with the endogenous isotope in the food matrix.

2.7.4 Double isotope labelling technique

This involves both intravenous administration of isotope trace element and intake of isotope labelled food. After the intake of extrinsic or intrinsic labelled food, a subject is given intravenous injection of a different tracer isotope through the antecubital vein (Sirichakwal *et al.,* 1985). *In vivo* determination of bioavailability is carried out by monitoring levels of the trace isotopes in urine and / or faeces by mass spectrometric methods. In this method, errors associated with endogenous secretion of the element are compensated by intravenous injection. Therefore, it is considered as the most accurate and reliable technique (Yang *et al*., 2005).

However, this technique suffers from errors associated with difference in natural abundance of the different tracer elements used, which is a major source of analytical bias estimated to be about 1.4 to 10% in selenium absorption (Sirichakwal *et al*., 1985).

2.8 Method of analysis of trace elements

Common modern methods used for analysis of trace elements in biological samples include neutron activation analysis (NAA), atomic absorption spectrophotometry (AAS), flame photometry (FP), mass spectrometry (MS), inductively coupled mass spectroscopy (ICP-MS), isotope dilution (ID), x-ray fluorescence (XRF) and proton induced x-ray emission (PIXE). In this study, flame atomic absorption spectrometry (FAAS) was used to analyse chromium, iron and zinc because it is cheap, simple and readily available. Hydride generation atomic absorption spectrometry (HGAAS) was used to analyse selenium because it is sensitive, versatile and available (Lujen, 1992; Skoog and Leary, 1992).

2.8.1 Atomic absorption spectrometry

Atomic absorption spectrometric methods involve analyte sample vaporization and conversion into atoms by flame, electrothermal furnace or laser radiation. Radiation from external source usually cathode hollow lamp of the same analyte is passed through the atoms vapour. The atoms absorb radiation and are promoted to excited states. The unabsorbed radiation passing through the cloud of atoms falls on a monochromator then onto a detector. Monochromator isolates the wavelength of interest and removes scattered light, while photo detector converts light signal exiting monochromator into electrical signal. The electrical signal is amplified and recorded by readout device as concentration. Incomplete atomization caused by low temperature or formation of heat-stable products in the flame, reduces the number of excited atoms therefore, depressing signal produced. The strength of absorption is related to the number of absorbing atoms as expressed by Equation 2.1

$$\mathbf{I} = \frac{\mathbf{\Pi eNF}}{\mathbf{MC^2}} \qquad \textbf{Equation 2.1}$$

But, $\frac{\Pi e}{MC^2}$ is constant, therefore I is directly proportional to NF

Where:

I = total amount of radiation absorbed

e- Electronic charge

F- Oscillators strength

C- Speed of light

N- Total number of atoms that can be absorbed

(Skoog and Leary, 1992).

2.8.2 Atomic spectra

Spectra obtained from gaseous atomic particles are made up of well defined narrow lines arising from electronic transitions of the outermost electrons. The transition of metals in these energy levels yields ultraviolet, visible and near- infrared radiations. In practical situations atomic spectra are rarely monochromatic or narrow, but broad and overlap due to chemical and physical changes taking place during atomization (Skoog and Leary, 1992).

2.8.3 Physical interference

Change in physical properties of flame and the analyte solution, such as viscosity, surface tension, temperature and vapour pressure lead to fluctuation in analyte signal thus increasing error margin of machine. However, this problem can be reduced by optimizing operating conditions of the machine (Lujen, 1992; Skoog and Leary, 1992).

2.8.4 Chemical interference

Chemical interference is caused by presence of heat stable products in the flame such as PO_4^{3-}, SO_4^{2-}, or AlO_2^{-}, during analysis of alkaline earth metals thus leading to depressed

signal from analyte. This problem may be minimized by operating the instrument at higher temperature or by adding releasing agents that have high affinity for interfering ion than analyte (Lujen, 1992).

Chemical interference is also caused by high rate of ionization of atoms in the flame at high temperatures. Whereas very high temperature reduces concentration of atoms in flame due to ionization, low temperature lowers atomization efficiency considerably leading to reduced signal. The effect of temperature is best illustrated by the Boltzmann distribution equation (equation 2.2), which describes the relative population of different states at thermal equilibrium;

$$\frac{N}{N_0} = g\, e^{\Delta E/kT}$$ **Equation 2.2**

Where N and N_0 are population of atoms at excited and ground state, respectively, E is the energy difference between the states, T is temperature in Kelvin and k is Boltzmann constant. Ionization interference can only be reduced by adding ionization suppressor, which provides a higher concentration of electrons to the flame to suppress ionization of analyte (Lujen, 1992).

2.8.5 Spectral interference

Presence of more than one absorption line in spectral band pass is a major interference in atomic absorption spectrometry, because it reduces sensitivity. There are many causes of spectral interference, but the major ones include presence of combustion products that exhibit broad-band absorption, and random movement of atoms in flame that cause Doppler effect. Other causes are collision of absorbing or emitting atoms with other atoms that result in pressure broadening and the uncertainty in finite amount of time that atoms spend in energy

levels between which transitions occur. This uncertainty is best expressed by Heisenberg's principle, which states that the shorter the lifetime of the excited state, the more uncertain is its energy relative to the ground. The Heisenberg's principle is described by equation 2.3.

$$\Delta E \delta t \geq \frac{h}{4\pi}$$ **Equation 2.3**

Where h is plank's constant, π is pi, and δ is change in time.

Change in energy (**ΔE)** is related to **hl / λ** and **1 / λ** is reciprocal of wavelength or wave number. Therefore, variation in energy results in variation in wavelength, a phenomenon that causes natural line broadening.

Where the source of spectral interference is the fuel and oxidant, then the effects may be corrected by use of blank readings. Spectral interference caused by known interferent can be minimized by adding an excess of interferent to both sample and standard. In electrothermal atomization, matrix interference is a major problem that can only be minimized by background correction (Lujen, 1992).

2.9 The AAS instrument

The AAS instrument consists of source of radiation, atomizer, monochromator, detector and read out system.

2.9.1 Sources of radiation

Sources of radiation used in AAS may be classified into two major groups, as line or continuous sources depending on band width of their wavelength. Continuous sources of radiation emit light over a broad spectrum of wavelength that makes them have less sensitivity. Examples of continuous sources of radiation include xenon arc, deuterium lamps

and mercury lamps. Line sources of radiation have bandwidths that are narrower than absorption peaks, therefore making them more sensitive than continuous sources of radiation. Examples of line sources of radiation include: hollow cathode lamps, electrodeless discharge lamps and laser beams (Skoog and Leary, 1992).

2.9.2 Atomisers for atomic spectroscopy

Atomisers provide high temperature source for desolvating and vapourising sample to obtain free atoms for spectroscopic analysis. There are two types of atomizers commonly used in atomic absorption spectroscopy, the flame atomiser and electrothermal atomizer. Laser atomiser is only used for specialized studies in research laboratory (Skoog and Leary, 1992).

2.9.3 Monochromators

Monochromators disperse or separate radiations so that the selected wavelength corresponding to a particular energy of interest is transmitted. Monochromator consists of lenses or mirrors that focus radiation, entrance and exit slit to restrict unwanted radiation and dispersing medium to separate wavelengths. There are two types of dispersing elements, prism and diffraction grating (Skoog and Leary, 1992).

2.9.4 Detectors

Detectors convert radiant energy into electrical signal. The common types of detectors include phototube, photomultiplier tube and photo diode arrays (Skoog and Leary, 1992).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study area

Busia District is one of the eight districts that form Western Province, Kenya. The district lies between 0° 1'36'' South and 0° 33' North and longitude 33° 54' 32'' west and 34° 25'24'' East. The district has two rainy seasons, long rains in March to May and short rains in September to October with a mean of 1550 mm per annum. The mean temperatures range from 22 to 30 ºC. Butula division is one of the six divisions in the district. Food crops grown in Butula division include maize, sorghum, sweet potatoes, soya beans, cowpeas, green grams, kales, sesame, cassava, avocadoes, bananas and oranges, while cash crops are sugar cane, coffee and sunflower. HIV prevalence rate in the district was 7.4% in 2002 (GOK, 2002).

3.2 Research design

This was a pre-intervention study on nutritional status of PLWHA and the potential use of indigenous food supplement on the management of HIV and AIDS. The study was carried out in three phases, the first phase involved baseline studies on clinical, nutritional and immunological status of PLWHA. In the second phase, levels of the trace elements were determined in selected indigenous foods and those found to contain high levels of the elements were used to make food formulation. In the last phase, bioavailability of the elements in the food formulation was estimated with *in vitro* digestion and results compared with algorithm estimates.

3.3 Study population

The study subjects were members of Rural Education and Economic Enhancement Programme (REEP), a local NGO operating in Butula division. The REEP seeks to improve the quality of life for marginalised groups in rural communities in Western Kenya. In the year 2005, membership of PLWHA in REEP was about 3,000 people. Through Community Health Workers (CHW), REEP supports the caregivers of PLWHA who are very sick, depressed or angry. Other activities of REEP include: reducing stigma of PLWHA within the community by creating forum for sharing experiences, initiating income generating activities, supporting orphan children with school fees problem, advocating for change in the widow inheritance custom, and supporting families of people dying with HIV and AIDS (Corbett, 2005).

3.4 Subjects sample size

Sample size for the study was determined according to the equation 3.1 (Nelson *et al*., 2004).

$$N = 2\sigma^2 \frac{\left(Z_{\frac{\alpha}{2}} + Z_\beta\right)^2}{d^2}$$ **Equation 3.1**

Where: $Z_{\frac{\alpha}{2}} = 1.96$ and $Z_\beta = 1.282$ (90% power of study).

$N = 2\text{x}\frac{10.51\sigma 2}{d^2}$, σ^2 = variable, d = difference to be detected: $u_1 - u_2 =$ **2.03 g/dL** serum,

$\sigma^2 = 2.8^2$. $N = 2\text{x}\frac{10.51\text{x}2.8^2}{2.03^2}$, $N = 40$. Therefore, 40 subjects were recruited per group. Three groups of the subjects were selected to participate in the study and 11 more subjects were added to take care of the natural attrition, bringing the total number of subjects to 131.

3.5 Recruitment of subjects

Purposive sampling method was used to recruit study subjects sequentially, especially the ones that were available and could easily access the REEP VCT centre. The selected potential subjects were briefed on the purpose, procedure of the study and keeping dietary record for the subsequent intervention study. Consenting subjects were requested to sign consent form.

3.6 Inclusion and exclusion criteria

The subjects were HIV-1 infected adult male and female aged 19 to 49 years visiting the local VCT centre. They were in clinical stage 1 or 2 of the CDC HIV and AIDS staging criteria. The subjects using ARVs, nutritional supplements, bed-ridden, hospitalized, expecting, lactating or not willing to give written formal consent were excluded from the study.

3.7 Baseline study

The baseline study was carried out to determine the feeding habits and nutritional status by use of a questionnaire. Blood samples were collected and used to analyse serum levels of zinc, CD4+ cell counts, CD3+ cell counts, CD8+ cell counts, and CD4+/CD8+ ratio of the subjects. Subjects were clinically examined and details of HIV associated opportunistic infections and BMI recorded by a physician from a local hospital. Subjects who required specialized medical attention were referred to appropriate medical facilities.

3.8 Ethical consideration and confidentiality

Clearance was sought from Ethical Review Committee of the Kenya Medical Research Institute (KEMRI) and research permit obtained from the Ministry of Education. Prior to the

study, informed voluntary consent of participants, hospital and REEP administrators were sought. Anonymity of the subjects was kept by giving them code numbers for confidentiality.

3.9 Study samples

3.9.1 Food samples, food sampling and pre-treatment

Food grains and tubers were bought in their natural form from a local market in Butula Division and packaged in a polythene bag. A portion of each food sample was withdrawn, sorted, cleaned with distilled water and dried in oven at 70 ºC for two hours. Dry samples were crushed into a fine powder using a mortar and a pestle and were put in polythene bags, labelled, sealed and kept at room temperature until analysed.

3.9.2 Blood samples, sampling and pre-treatment

A registered nurse took 10 ml of blood from subjects by vein puncture in the left hand of the subjects in sitting position by use of propylene stylus catheter. Portions of 2 ml blood sample for CD3+, CD4+, and CD8+ cell counts analysis were processed within 24 hours. Portions of 5 ml blood sample used for analysis of trace elements were allowed to clot for 30 minutes and centrifuged at 3,000 revolutions per minute for 20 minutes. Serum was transferred using pipette tip into acid washed vials and stored at below 4 ºC. Portions of 3 ml blood sample for other analyses were preserved with EDTA and stored in acids washed polypropylene vials below -20 ºC.

3.10 Bioavailability studies

Indigenous food grains with high levels of zinc, iron, chromium and selenium identified from chemical analysis were used to prepare locally acceptable food products. The proportions of selected foods were made to maximize the levels of the elements in the food supplements. Foods grains used in this study included finger millet, sesame and pumpkin seeds.

3.11 Reagents and apparatus

Glassware was washed with detergent, rinsed with distilled water and maintained in a hot bath with 50% v/v HNO_3 for a period of 1 hour, rinsed with distilled water and oven dried. Dialysis tubing (mm cut off 10 kD) was washed three times with distilled water and soaked overnight in 0.1 M $NaHCO_3$. Standard reagents were analar grade (zinc metal, iron metal, K_2CrO_4, SeO_2). Other chemicals used include pepsin, pancretin, NaOH, $NaHCO_3$, HNO_3, $NaBH_4$, H_2SO_4, HCl, H_2O_2, $HClO_4$, EDTA and triton X-100, which were of analar grade. All reagents used were of analytical grade and were purchased from local chemical stores.

3.12 Instruments

Buck Scientific Company (model 210 VGP) FAAS was used for analysis of zinc, iron, calcium and chromium, whereas HGAAS apparatus (VARIAN, Model Spectra AA – 10/20) was used for analysis of selenium**.** The operating parameters for the working elements were set as recommended by the manufacturer. A questionnaire was used to collect information on feeding habits, clinical and nutritional status of the subjects (Appendix 14 to 16).

3.13 Experimental procedure

3.13.1 Preparation of stock solution and other reagents

Procedure given by Van Loon (1990) was used to prepare stock solutions from standard analar reagents. A series of calibration standards was prepared by diluting the stock solutions with distilled water. Pepsin suspension was prepared by suspending 16 g of pepsin powder in 100 ml of 0.1 M HCl and pancretin-bile extract was prepared by dispersing 4 g pancretin and 25 g bile extract in 1 L of 0.1 M $NaHCO_3$.

3.13.2 Method validation

Two types of quality control approaches were used to check the determination of trace elements; namely, recoveries of the trace metals added to pumpkin seeds and precision by repeated determinations (6 times) of trace metals content in the pumpkin seeds. Aliquots of 25 ml of 1 ppm (chromium), 1.5 ppm (zinc) and 7 ppm (iron) were added to 1 gram of sub-sample of food and digested separately as other food samples and the procedure was carried out in triplicates. Levels of the elements were determined in the triplicates of digest and values obtained used to determine percentage recovery. The entire procedure was also carried out for all blank samples containing the same amount of acids. Linearity of the method was checked by use of regression equation and plotting calibration curve of the standard readings against concentrations.

3.13.3 Food sample extraction

One gram of each sample was weighed using analytical balance and placed in 500 ml Kjeldahl digestion flask. Nine millilitre and 4.5 ml of concentrated HNO_3 and H_2SO_4, respectively were added to the sample in digestion flask. The mixture was heated gently in

the digestion block until brown fumes disappeared, then allowed to cool for about 15 minutes. A portion of 1.5 ml of perchloric acid was added to the cool mixture and heated until the solution turned clear and white fumes seen. The resulting mixture was left to cool, then filtered into 50 ml volumetric flask and topped to the mark with distilled water. The final solution was transferred into acid cleaned plastic bottle, sealed and stored in a deep freezer awaiting analysis. The blank solution was prepared by mixing 9 ml, 4.5 ml and 1.5 ml of nitric, sulphuric and perchloric acid (ratio of 6:3:1), respectively and treated as samples. All the food samples were analysed in triplicates and results reported as mean ± standard deviation.

3.13.4 Serum sample

Serum was diluted into 1:10 ratio with distilled water and analyzed according to procedure given by Jian-Xin (1990).

3.13.5 Immunological status

Flow cytometry was used to determine CD3+T, CD4+T and CD8+T cells count and CD4+/CD8+ cells ratio in 2 ml of the blood, all the analyses were done at KEMRI.

3.13.6 Nutrients in vitro bioavailability studies

3.13.6.1 Pepsin digestion

One gram of fine ground food formulation sample was placed into 200 ml plastic bottle and mixed with 4 ml distilled water. Pepsin suspension of 2.25 ml was added to the mixture and pH was adjusted to 2 using 6 M HCl. Contents of the mixture in the bottle were brought to

100 ml using distilled water. The bottle was incubated at 37 ºC for 2 hours, shaking in water bath and pH of 2 maintained by adding HCl. At the end of incubation, a duplicate of 20 g of digest was transferred into 250 ml Erlenmeyer flasks. Titrable acidity was determined on 20 g aliquot of pepsin digest to which 5 ml of pancretin-bile extract was added and calculated as amount in moles of 0.2 M sodium hydroxide required to attain pH of 7.0.

3.13.6.2 Pancretin digestion

Segments of dialysis tube containing 25 ml of distilled water and amount of $NaHCO_3$ equivalent in moles to the NaOH needed to neutralise the pepsin digest were placed in each Erlenmeyer flasks containing the pepsin digest. The mixture was incubated at 37 ºC with shaking for 30 minutes or longer until the pH reached 5. Pancretin-bile extract of 5 ml was added to the mixture in the flask and incubation continued for 2 hours or longer until pH of the digest reached 7. At the end of pancretin digestion (pH = 7), segments of dialysis tubes were removed and volume of dialysate and weight of retentate measured. Levels of trace elements in the dialysate were analysed and dialysis percentages calculated as follows: dialysis % = 100 x D/C, where D = iron content of the dialysate (l g sample) and C = total iron content of the sample (l g sample). The procedure was repeated with a blank sample (distilled water).

3.13.7 Nutrients algorithms bioavailability studies

3.13.7.1 Estimation of the absorption of iron

Mathematical model for iron absorption developed by Hallberg and Hulthěn (2000) was used to estimate percentage of bioavailable iron. The model is based on absorption of iron from a wheat roll (basal absorption 22.1%) without inhibitors or enhancers and adjusted to a

reference dose absorption of 40.0 %. The reference dose absorption of 40.0 % corresponds to a serum ferritin concentration of 23 µg/L. The basal absorption is multiplied by factors which affect absorption that include phytate, calcium and ascorbic acid. The model was used to estimate bioavailable iron in millet grains, sesame and pumpkin seeds as given in Equation 3.2.

$$\text{T}otal\ bioavailable\ iron\ = factor\ of\ phytate\ \times\ factor\ of\ ascorbic\ acid\ \times\ factor\ of\ calcium\ \times\ basal\ factor\ of\ 22.1$$ **Equation 3.2**

Phytate, ascorbic and calcium factors were computed using equations 3.3, 3.4 and 3.5, respectively.

3.13.7.1.1 Effect of phytate

Phytate reduces iron absorption in a dose dependent manner; the inhibitory effect of phytate is obtained by determining the antilogarithm of Equation 3.3.

$$Log\ absorption\ ratio\ (with\ or\ without\ phyate) = -0.30 \times log(phytate\ P + 1)(in\ mg)$$

Equation 3.3

3.13.7.1 .2 Effect of calcium

Calcium reduces iron absorption when present in food in the range of 50 to 600 mg as described by the equation 3.4.

$$Iron\ absorption\ (mg) = 0.4081 + [\frac{0.5919}{1+10^{-[2.022-\log(Ca+1)]\times 2.919)}}]$$ **Equation 3.4**

3.13.7.1 .3 Effect of ascorbic acid

Ascorbic acid enhances on iron absorption in a dose dependent manner and its effects are influenced by phytate concentration in food. Equation 3.5 describes the effect of ascorbic acid on absorption of iron.

$$Absorption\ ratio = [1 + 0.01AA\ (in\ mg) + \log[phytate - p\ (in\ mg)] \times 0.01 \times 10^{0.8875 \times \log(AA + 1)}$$

Equation 3.5

3.13.7.2 Estimation of the absorption of zinc

Algorithm developed by Miller *et al.* (2007) that models zinc absorption as a function of amount of phytate and zinc in food was used to estimate amount of bioavailable zinc in the food as given in Equation 3.6.

$$TAZ = 0.5 \left\{ A_{max} + TDZ + K_R \cdot \left(1 + \frac{TDP}{K_P}\right) - \sqrt{\left(A_{max} + TDZ + K_R \cdot \left(1 + \frac{TDP}{K_p}\right)\right)^2 - 4.A_{max} + TDZ} \right\}$$

Equation 3.6

The equation describes a saturable, carrier-mediated trivariate model with two independent variables: TDZ and TDP, where: TDZ is total dietary zinc (millimoles) and TDP is total dietary phytate (millimoles) in food. The dependent variable is total absorbed zinc (TAZ) in millimoles, while the three parameters of the model include: maximum zinc absorbed (A_{max}), which is related to the number of zinc transport receptors, equilibrium dissociation constant of zinc-phytate complex (K_P) and equilibrium dissociation constant of zinc receptor complex (K_R). The values of A_{max}, K_P and K_R used in the model are 0.11, 0.06 and 1.46 respectively and were obtained from estimates of Hunt *et al.* (2008) in a low zinc bioavailability diet.

3.13.7.3 Algorithms for estimating bioavailable selenium

Electronic data search was done in the internet using the selection criteria: radioactive or stable isotope studies that estimated selenium absorption and retention from food, studies that used diets with exogenous selenium salts were excluded. Data collected were subjected to multiple linear regression analysis, where selenium absorption (%) was regressed against dietary selenium, pre-study serum selenium and the duration of intake of the diet.

3.13.8 Administration of questionnaire

A physician in the local hospital administered the questionnaire on clinical evaluation and determination of BMI of the subjects, while research assistants trained by a dietician administered the questionnaire on feeding habits, socio-economic status and nutritional status. The BMI was determined according to the procedure given by RCQHC (2005b), whereas the nutritional status and feeding habits were estimated based on interview response on the diet composition, number of meals per day, food sources and accessibility. The socio-economic status was evaluated using interview response on marital status, source of income and level of education of the subjects.

3.13.9 Data analysis

Statistical analyses were done using SPSS for windows version 11.5. Experimental data were expressed as mean ± standard deviation and reported in tables. Differences in the groups were determined by one way ANOVA and was considered significant when $p < 0.05$. Relationship between subjects immunological status (CD4+ and CD8+ cell counts and CD4+/CD8+) and haematological status (MCHC, MCH, RDW and HGB). Incidence of opportunistic diseases was assessed using Pearson correlation co-efficient and the association was considered to be

significant at $p < 0.05$. Linear regression analysis was used to model selenium absorption as a function of total dietary selenium, pre-study serum selenium and duration of intake of selenium supplement. Zinc and iron bioavailability in the foods were calculated using the Microsoft Excel programme (version, 2007) and results presented in tables.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Introduction

A total of 131 PLWHA in Butula Division participated in the study, where information on age, gender, feeding habits, nutritional status and immunological status were collected. Twenty four indigenous foods that are widely consumed in Butula Division were collected from Butula market and analysed in triplicate, for levels of iron, zinc, selenium and chromium. The food samples that were found to contain sufficient levels of the elements were used in preparation of food formulation and the bioavailability of nutrients estimated by use of algorithms and *in vitro* digestion model. The results are presented and discussed in the following sections.

4.2 Personal characteristics Feeding habits and nutritional status studies

Personal characteristics, feeding pattern and nutritional status and feeding habits of the subjects were determined using a questionnaire. The results are presented and discussed in the following subsections.

4.2.1 Personal characteristics

The information on personal characteristics of the study subjects collected include: age, intake of substances (smoking and alcohol) and nutritional supplement and marital status. The results are presented and discussed as follows.

4.2.1.1 Age

The age was determined using date of birth of the subjects and only 128 subjects gave information on their date of birth. Table 4.1 shows age of the subjects.

Table 4.1 The mean and range of age of male and female subjects

Parameter	Male (n = 17)		Female (n = 111)		P = value
	Mean	Range	Mean	Range	
Age (years)	42.41	25-68	38.17	20-91	0.064

The female PLWHA that participated in the study were younger (38.17 years) than the male PLWHA (42.41 years). However, age of the female subjects was not significantly different from the male subjects (p = 0.064). Age of the female subjects was ranging from 20 to 91 years of which 9 were above 49 years old, whereas the male subjects ages ranged from 25 to 68 years of which 4 were above 49 years old.

4.2.1.2 Substance intake

The data on smoking status, intake of alcohol and use of nutritional supplements were obtained from only 128 subjects and are presented in Table 4.2.

Table 4.2: Frequency and percentage of PLWHA smoking, taking alcohol and nutritional supplement

Parameter	Male (n = 17)		Female (n = 111)		p = value
	Frequency	Percentage (%)	Frequency	Percentage (%)	
Smokers	1	5.8	2	1.8	0.338
Use of alcohol	2	13.3	5	4.4	0.253
Use of food supplement	0	0.0	4	3.6	0.520

A low proportion of the subjects smoked (men = 5.8 %, women = 1.8 %) and took alcohol (men = 13.3 %, women = 4.4 %). This is encouraging because, smoking and intake of alcohol are known to increase oxidative stress in the body that leads to suppressed immune system (Simonetti *et al*., 1993; Simonetti *et al*., 1995; Pryor and Stone, 1993; Eiserich *et al*., 1995). Alcohol and smoking are also associated with reduced absorption of antioxidant nutrients, such as vitamin C that play key role in immune system (Pelletier, 1997). Therefore, PLWHA need to be discouraged from smoking and taking alcohol, as these habits could lead to faster progression of HIV infection to AIDS.

A small percentage of the female subjects (3.6 %) used food supplements that were made from the local food materials, which were then used to prepare food supplement in this study. Low use of the food supplements indicate that, majority of PLWHA were neither aware of the importance of food supplement in meeting the increased nutritional requirements associated with HIV and AIDS, nor could afford food supplements that are in the market.

4.2.1.3 Marital status

Data on the marital status of PLWHA in Butula division were obtained from only 28 subjects and are recorded in Table 4.3.

Table 4.3: The frequency and percentage of marital status of the

Parameter	Male (n = 5)		Female (n = 23)	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Single	3	60.0	0	0.0
Monogamous marriage	1	20.0	6	26.1
Polygamous marriage	0	0.0	2	8.7
Widowed	1	20	10	43.5
Other	0	0.0	6	26.1

The highest proportion of the male subjects were single (60 %), whereas highest proportion of the female subjects were widowed (43.5 %). Proportion of monogamous marriage was modest in both male (20.0 %) and female (26.1 %) subjects and there was no single female subject. No polygamous and other types of marriages except monogamous and polygamous observed among the male subjects, but 8.7 % and 26.1 % of the female subjects were in polygamous and other types of marriages, respectively. Almost a half of the subjects (49 %) did not have a spouse (widowed or single).

4.2.2 Feeding pattern and sources of food

Information on the feeding pattern and sources of food of the subjects were obtained from 129 and 71 subjects, respectively and are presented in Table 4.4.

Table 4.4: The percentage and frequency of feeding pattern and sources of food of subjects

	Parameter	Frequency	Percentage %
Number of meals per day	1	17	13.0
	2	79	60.3
	3	30	22.9
	4	3	2.3
Source of food	Buy	25	20.3
	Farm	14	60.2
	Buy and farm	21	17.1
	Other	11	2.4

Majority of the subjects were taking less than three meals a day: 60.3 % (two meals/day) and 13.0 % (one meal/day), whereas a small proportion was taking three meals per day (22.9 %) and four meals per day (2.3 %). Furthermore, most subjects did not take meals regularly (58.8 %) and only 39.7 % reported taking meals regularly. The PLWHA require at least three meals per day, with snacks between the meals in order to meet the increased energy and nutrients demand caused by HIV infection (GOK, 2006).

The major source of food for PLWHA in the study was farm (60.2 %), whereas market contributed a modest proportion (20.3 %). Less than a half of the subjects (17.1 %) obtained their food from both market and farm, and a small proportion (2.4 %) relied on other food sources such as donation. This indicates that most subjects (97.6 %) were food secure because they obtained food from their farms and could afford to buy what was not produced from their farm. This is encouraging because, food insecurity among PLWHA leads to migratory livelihood strategies and high risk sexual behaviours, which increase risk of HIV

re-infection (NASCOP, 2005). The HIV re-infection with a different strain increases HIV virulence, resulting in faster progression of HIV and AIDS (Stine, 2005).

4.2.3 Food accessibility

Information on food accessibility by the subjects was obtained by asking the subjects whether there are foods they should be consuming, but are not accessible and reasons for not accessing the foods. A total of 52 and 131 subjects gave information on foods not accessible and reasons for not accessing the foods, respectively. The results are presented in Table 4.5.

Table 4.5: Frequency and percentage of the subjects accessing foods and reasons for not accessing foods

Parameter		Frequency	Percentage %
Are there foods not accessible	Yes	49	94.2
	No	3	5.8
Reasons for not accessing foods	Cannot afford	121	92.4
	Infertile shamba	1	0.8
	Not available	4	3.0
	Children do not eat	1	0.8
	Other	4	3.0
	Total	131	100

A majority of the subjects (94.2 %) were not accessing foods they thought they should be consuming because they could not afford them (92.4 %). In addition, a small proportion could not access foods due to: infertile shamba (0.8 %), the food not available (3.0 %) and other reasons (3.0 %). A small proportion (5.8 %) was accessing foods they thought they should be eating.

Over a half of the total subjects (60.2 %) was food secure, because they rely on their farms as major source of food. A majority (92.4 %) had narrow range of food choices, because, they could not afford foods grown elsewhere, which they thought could be beneficial to their health. Therefore, alternative local foods with high levels of the essential elements need to be identified and the subjects encouraged to grow them.

4.2.4 Feeding habits

Information on the household food composition was collected and presented as percentage of the major types of foods in Figure 4.1.

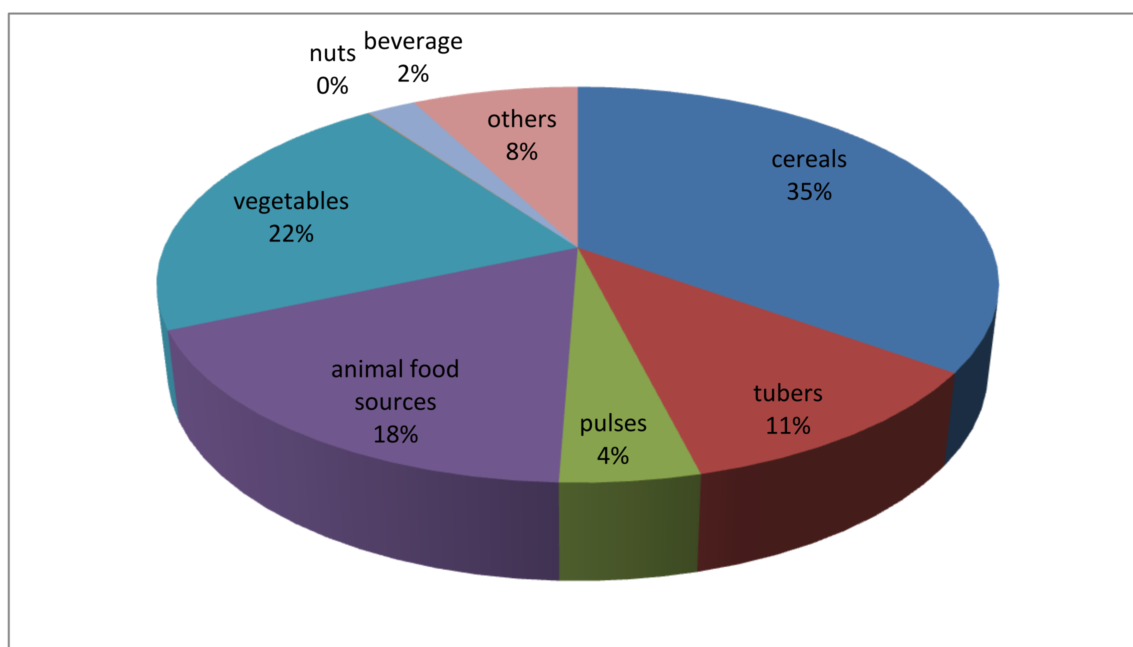

Figure 4.1 Percentage of major foods taken by the subjects

Cereal foods were widely consumed (35%), with nuts being the least consumed foods (< 1 %). Starch based foods (cereals and tubers) accounted for (46 %) and protein sources of food

(pulses and animal sources of food) accounted for 22 % of foods consumed by the subjects. Plants food sources (nuts, cereals, pulses, vegetables, beverage and tubers) constituted 74 % of the total food sources.

Cereals and tubers, which are major sources of carbohydrates constituted 35 % and 11 %, respectively of diet of the subjects. This indicates possible adequate intake of carbohydrates by the subjects. There is need for high intake of carbohydrates, which are major sources of energy because, PLWHA have higher energy requirements depending on the stage of the HIV and AIDS. The asymptomatic and symptomatic PLWHA require between 10 to 15 % and 40 to 50 %, respectively, above the energy requirement of a normal healthy person (Woods, 1999). Although bioavailability of trace elements is low in cereals and tubers, they are major sources of the trace elements in developing countries, because they form the bulk of foods used in these countries (WHO/FAO, 2004). Bioavailability of the trace elements in the cereals and tubers can be improved by simple traditional food preparation practises, such as fermentation, milling, soaking, germination, roasting and appropriate food combinations (Walingo, 2009).

Although vitamin levels are affected by food preparation practises, the modest proportion of vegetables in the diet (22 %), which are their major sources, indicates possible adequate intake. Vegetables are also good sources of minerals, trace elements and dietary fibres. Vitamins and trace elements have antioxidant activities that remove reactive oxygen species that occur at high levels among PLWHA, while dietary fibre enhances bowel movement and overall health of the digestive system (GOK, 2006). However, high levels of fibre, phytate and polyphenols in vegetables may reduce absorption of the trace elements, therefore,

vegetables need to be taken in moderate amounts (Fredlund *et al*., 2006; Matuschek and Svanberg, 2005; Hallberg and Hulthèn, 2000).

The proportions of pulses and animal food sources in diet of the subjects were low (4 and 18 %, respectively) indicating possibly, low intake of proteins. The PLWHA have increased requirements of protein (2 × RDA), because protein is used in the production of antibody, new cell and wound healing (Woods, 1999; Roubenoff and Laura, 1999). Protein food sources contain high levels of some trace elements and enhance absorption of the trace elements from other food sources (Hurrel, 2003). Therefore, the subjects need to increase proportion of pulses and animal food sources in their diet, to meet the increased protein requirements and enhance absorption of the trace elements.

4.3 Immunological and nutritional status

The health condition of the subjects was determined by the assessment of BMI, immunological and clinical status.

4.3.1 The CD3+, CD4+ and CD8+ cells counts and CD4+/CD8+ ratio

The CD3+, CD4+ and CD8+ cells counts and CD4+/CD8+ ratio were used in assessing immunological status of the subjects. A total of 103 subjects participated in the immunological study and results are given in Table 4.6.

Table 4.6: The mean ± standard deviation and range of CD3+, CD4+ and CD8+ cells counts and CD4+/CD8+ ratio of the subjects

Parameter	Male (n = 16)		(Female n = 87)		P = value
	Mean ± SD	Range	Mean± SD	Range	
CD3+ (cells/mm^3)	1579.8±498	709-2821	1870±770	674-5431	0.150
CD4+ (cells/mm^3)	725±396	148-1315	794±466	98-2250	0.577
CD8$^+$ (cells/mm^3)	751.9±403	179-1572	979.5±685	226-4760	0.202
CD4$^+$/CD8$^+$	1.483±1.32	0.2-4.5	1.187±0.97	0.1-3.8	0.293

The mean levels of CD3+ (1870 cells/mm^3), CD4+ (794 cells/mm^3) and CD8+ (979.5 cells/mm^3) observed in the female subjects were slightly higher, compared to the mean levels of CD3+ (1579.8), CD4+ (725) and CD8+ (751.9) cells/mm^3 observed in the male subjects. The highest mean level of CD4/CD8+ (1.483) was observed in the male subjects, while the lowest mean level of 1.187 was observed in the female subjects. The levels of CD4/CD8+ were in the range of 0.1 to 3.8 and 0.2 to 4.5 in the female and male subjects, respectively. The overall mean levels of CD3+ (1579.81 cells/mm^3), CD4+ (725.8 cells/mm^3) and CD4+/CD8+ (1.483) indicate that the subjects did not have significant immunosuppresion, which usually occurs at CD4+ and CD3+ cells below 500 and 1000 cells/mm^3, respectively (WHO, 2005; Chapel and Sewel, 1990). One way ANOVA test showed no significant difference in CD3+ and CD8+ cells and CD4+/CD8+ ratio between the male and female subjects. Therefore, the female and male subjects had similar immunological status at baseline. Level of CD4$^+$ cell count was used to determine the stage of HIV and AIDS disease among PLWHA in Butula Division. The frequency, percentage (%) and range of CD4+ cell count are given in Table 4.7.

Table 4.7: The frequency, percentage and range of CD4+ cell count of the subjects

CD4+ (cells/mm^3)	Male (n = 16)		Female (n = 87)	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Below 200	1	6.3	7	6.9
200-500	6	37.5	19	21.8
Above 500	9	56.3	62	71.3

The highest proportion of both male (56.3 %) and female (71.3 %) subjects had CD4+ cells above 500 cells/mm^3, whereas the lowest proportion of both male (6.3 %) and female (6.9 %) subjects had CD4+ cells below 200 cells/mm^3. Only modest proportion of both male (37.5 %) and female (21.8 %) subjects had CD4+ cells ranging from 200 to 500 cells/mm^3. The CD4+ cells of majority of the subjects (male = 56.3 %, female= 71.3 %) were above 500 cells/mm^3, indicating that they did not have significant immunological suppression. Less than a half of all the subjects (male = 37.5 %, female = 21.8) had moderate immune suppression (CD4+ cells ranging from 200 to 500). Although low CD4+ cells (below 200) associated with severe immune suppression were observed in a very small proportion of subjects (male = 6.3 % and female = 6.9 %), some PLWHA had low CD4+ cells but were healthy, therefore clinical observation is used to confirm immunological status (WHO, 2005).

4.3.2 Clinical status

Clinical status of PLWHA was evaluated by assessment of opportunistic infections, weight loss, fatigue, lack of appetite and fever. A total of 126 subjects participated in the clinical status study and results presented in Table 4.8.

Table 4.8: The percentage and frequency of opportunistic infections, fatigue, fever and lack of appetite among the subjects

Parameters	Male (n = 16)		Female (n = 110)		All subjects (n = 126)		p =
	Frequency	Percentage %	Frequency	Percentage %	Frequency	Percentage %	
Malaria	2	12.5	32	29	34	27	0.173
Fatigue	7	43.8	56	51.0	63	50.0	0.573
Lack of appetite	2	12.5	23	21.3	25	20.0	0.365
Kaposis Sarcoma	1	6.3	0	0	1	0.8	0.012
Lympha-demopathy	5	31.3	6	5.5	11	8.7	0.001
Herpes zooster	1	5.9	4	3.7	5	4.0	0.673
Pallor	2	12.5	17	15.6	19	15.1	0.899
Pneumonia	2	11.8	17	15.6	19	15.1	0.684
URTI	4	25	37	33.6	41	32.5	0.481
Diarrhoea	1	5.9	6	5.5	7	5.6	0.950
Fever	3	18.8	22	20.0	25	19.8	0.868
Weight loss	10	62.5	43	39.1	53	42.1	0.151

Low proportion of the subjects had malaria (27 %), lack of appetite (20.0 %), Karposis Sarcoma (0.8 %), lymphademopathy (8.7 %), herpes zooster (4.0 %), pallor (15.1 %), pneumonia (15.1 %), diarrhoea (5.6 %) and fever (19.8 %), which are common in insignificant immune suppression (CD4+ = 725.8 cells/mm^3) observed, indicating that the

subjects were in stage 1 and 2 of HIV and AIDS according to CDC staging criteria (WHO, 2005). About a half of the subjects had fatigue (50.4 %), less than a half had weight lose (42.1 %) and upper respiratory tract infection, URTI (32.5 %). There was no significant difference between female and male subjects in weight loss, lack of appetite, fever and other opportunistic diseases, except in Kaposis Sarcoma ($p = 0.012$) and Lymphademopathy ($p = 0.001$).

The HIV infection increases resting energy expenditure by 10 %, while secondary infections increase energy requirements by 29 %. When the increased energy requirements are not balanced by increased caloric intake, the PLWHA suffer from weight loss and fatigue (Macallan, 1999). Therefore, weight loss (42.1 %) and fatigue (50.4 %) observed among the subjects could be due to imbalance between energy requirements and intake. The weight loss could be reversed by increasing energy intake by 40 to 50 % over the usual requirements (Woods, 1999). Secondary infections also increase oxidative stress and rate of cellular turnover of the immune system, resulting in depletion of immunity boosting nutrients, such as vitamins and trace metals (Fracker, 1994). Therefore, the subjects need to increase intake of calories, vitamins and the trace elements.

4.3.3 Nutritional status

Nutritional status of the subjects was determined using BMI and haematological indices of the subjects. A total of 128 subjects participated in nutritional status study and the results are presented and discussed in the following subsections.

4.3.3.1 Body mass index (BMI)

Height and weight of the PLWHA were measured and results used to compute BMI. The range, frequency and percentage of BMI are given in Table 4.9.

Table 4.9: The frequency and percentage of BM classes of male and female subjects

BMI classes	Male (n = 17)		Female (n = 111)	
	Frequency	Percentage	Frequency	Percentage
Under weight 18.5	4	23.5	12	10.8
Normal weight 18.5-24.9	11	64.7	88	79.3
Overweight 25- 29.9	0	0.0	9	8.1
Obese over 30	2	11.8	2	1.8

Majority of the male (64.7 %) and female (79.3 %) subjects were within normal weight range of a healthy adult person, whereas 10.8 % of females and 23.5 % of males were underweight. Obese males and females were 11.8 % and 1.8 %, respectively, while 8.1 % of female subjects were overweight. The BMI data indicates that, majority of PLWHA that participated in the study ate adequate food and were not having metabolic abnormalities, oral and gastrointestinal complications that limit food intake and absorption, which associated with HIV-wasting and occur in the symptomatic stage of HIV and AIDS (GOK, 2006). Therefore, PLWHA that participated in the study had normal weight because they were at asymptomatic stage of HIV and AIDS.

The PLWHA that are underweight (BMI<18.5) are encouraged to increase food intake because, underweight is associated with energy deficit and low CD4+ cells, therefore, they are at risk of high rate of progression of HIV into AIDS (Macallan, 1999; Hogan *et al.*,

2003). The overweight and obese usually have low antioxidants such as β- carotene and vitamin C, therefore, are at risk of high oxidative stress that suppresses immunity, resulting in faster progression of HIV into AIDS (Galan *et al*., 2005; Allard *et al*., 1998; Favier *et al*., 1994).

4.3.3.2 Haematological status

The data on iron status of PLWHA in the study was obtained by analysis of levels of the mean corpuscular volume (MCV), red blood cell (RBC), haemocrit (HCT), haemoglobin (HGB), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red blood cell distribution width (RDW) in the blood. Table 4.10 shows mean and range of MCV, RBC, HGB, MCH, MCHC and RDW.

Table 4. 10: The mean and range of MCV, RBC, HGB, HCT, MCHC and RDW in PLWHA

Iron status indicator	Males (n = 17)		Females (n = 111)		P = value
	Mean	Range	Mean	Range	
HGB (g/dL)	11.34	7.7 - 13.90	11.22	6.2 - 15.90	0.809
RBC (cells counts) $x10^6/\mu L$	4.30	2.56 - 5.39	4.16	2.86 - 5.59	0.338
MCV (fL)	88.84	68.7 – 103.40	89.80	27.3 - 123.20	0.741
MCHC (g/dL)	29.74	28.4 – 31.10	29.98	26.1 – 32.70	0.325
MCH (pg)	26.42	20.1 – 30.50	27.13	17.9 – 38.90	0.435
HCT (%)	38.10	26.4 – 46.80	37.40	23.7 – 51.60	0.631
RDW %	15.20	12.2-20.00	15.30	11.8-30.40	0.333

The male subjects had highest mean levels of HGB (11.34 g/dL) that ranged from 7.7 to 13.90 g/dL, compared to those in female subjects (11.22 g/dL) that ranged from 6.2 to 15.90

g/dL. The highest level of RBC was observed in the male PLWHA (4.30 cells counts $x10^6/\mu L$) that ranged from 2.56 to 5.39 cells counts $x10^6/\mu L$, whereas the female PLWHA had the lowest level of RBC (4.16 cells counts $x10^6/\mu L$) that ranged from 2.86 to 5.56 cells counts $x10^6/\mu L$. The female subjects had the highest mean level of both MCV (89.80 fL) and MCHC (29.98 g/dL) that ranged from 27.3 to 123 fL and 26.1 to 32.70 g/dL, respectively. On the other hand, the male subjects had the lowest mean levels of MCV (88.84 fL) and MCHC (29.74 g/dL) that ranged from 68.7 to 103.4 fL and 28.4 to 31.1 g/dL, respectively. The mean level of MCH observed in the female subjects (27.13 pg) was higher than the mean level observed in the male subjects (26.429 pg). Levels of MCH in the male and female subjects ranged from 17.9 to 38.9 pg and 20.1 to 30.5 pg, respectively. The male subjects had the highest level of HCT (38.1 %) that ranged from 26.4 to 46.5 %, whereas the female subjects had the lowest mean level of HCT (37.4 %) that ranged from 23.6 to 51.6 %. The highest mean level of RDW (15.3 %) was recorded in the female subjects, compared to level in the male subjects (15.2 %) and was in the range of 11.8 to 30.4 % and 12.2 to 20.0 %, respectively.

Levels of MCV (89.8 and 88.8 fL) and RBC (4.3 and $4.16\times10^6/\mu L$) observed were above the cut off values of MCV (89 and 81.0 fL) and RBC (4.3 and $3.8\times10^6/\mu L$) for anaemia in males and females, respectively (UNCF/UNU/WHO, 2001). However, low levels of MCHC (29.7 and 29.98 g/dL) and high levels of RDW (15.2 and 15.4 %) observed are indicative of anaemia, which are set below 32 g/dL (MCHC) and above 15.0 % (RDW) for adult person (UNCF/UNU/WHO, 2001; CDC, 1998).

Low levels of HGB in the males (11.3 g/dL) and females (11.2 g/dL) also showed anaemia in males and mild iron deficiency in females, based on the cut-off of below 12 g/dL as

recommended by UNCF/UNU/WHO (2001). Presence of anaemia in the male subjects was further confirmed by levels of haemocrit (38.1%) that were lower than the cut off levels for anaemia (39.9 %) (CDC, 1998). The different interpretations of the parameters confirm that, they are not specific and sensitive; therefore, more than one parameter needs to be used in determining iron status (Hambidge, 2003).

Levels of haemoglobin were used to determine percentage prevalence of anaemia and severe anaemia between the gender and results are presented in Table 4.11.

Table 4.11: The percentage of the subjects with severe anaemia and anaemia

Anaemia prevalence	Male (n = 17)		Female (n = 111)	
	Frequency	Percentage %	Frequency	Percentage %
Severe anaemia (HGB < 7 g/dL)	0	0.0	3	2.7
Anaemia (HGB 7- < 12 g/dL)	8	47.0	67	60.4

Severe anaemia was not observed among the male subjects (0 %) but, 2.7 % of the female subjects had severe anaemia. Majority of the female subjects (60.4 %) and almost half of the male subjects (47.0 %) had anaemia. The overall prevalence rate of severe anaemia and anaemia among the subjects was 2.3 % and 60.9 %, respectively. The HGB levels vary with altitude, smoking status and race (CDC, 1998). Levels of HGB increase by 0.2 g/dL among people living in high altitude areas (1000 m above sea level) for a long time, such as in the study area, where altitude ranges from 1130 to 1375 m above sea level (UNCF/UNU/WHO, 2001; GOK, 2002). Black Africans HGB levels are lower than those of other races by 1.0

g/dL (UNCF/UNU/WHO, 2001). When the effects of altitude (0.2 g/dL) and race (1.0 g/dL) are applied to the study subjects, the cut off levels for severe anaemia and anaemia would be 6.2 g/dL and 11.23 g/dL, respectively. Based on the adjusted cut-off levels no subjects had severe anaemia, whereas 35.3 % male and 45.0 % female subjects had anaemia.

Although the use of haemoglobin levels in detection of iron deficiency anaemia may be flawed, because the levels vary with seasons, such as menstrual cycle and HGB levels drop when iron deficiency is already severe (Lynch, 2004). The HGB levels are still useful and recommended for defining anaemia and iron deficiency in developing countries, where resources limit use of other parameters that require expensive equipment. Presence of anaemia sometimes may not be due to iron deficiency because, there are also other causes of anaemia, such as pathological disorder like alchlorhydine and bleeding, and deficiency of folic acid, riboflavin, vitamin A and B_{12}. However, in the developing countries where iron deficiency causes over a half of anaemia, prevalence rate of anaemia is still a useful clinical, experimental and epidemiological criterion for defining severity of iron deficiency (UNCF/UNU/WHO, 2001; Lynch, 2004). According to UNCF/UNU/WHO (2001) classification, the prevalence rate of anaemia in the male subjects (35.3 %) is moderate (above 30 %), while that in the female subjects (45.0 %) is severe (above 40 %), and therefore requires nutritional intervention.

Positive significant correlations were observed between CD4+ cells and MCH ($r = 0.262$, $p < 0.01$), CD4+ cells and MCHC ($r = 0.243$, $p < 0.05$), CD4+ cells and HGB ($r = 0.188$, $p = 0.009$) and CD8+ cells and RDW ($r = 0.315$, $p < 0.01$). Negative significant correlations were also observed between CD4+ cells and RDW ($r = -0.259$, $p = 0.01$) and CD8+ cells and MCH

(r = 0.235, p < 0.05). The correlation between haemoglobin and CD4+ cells among the subjects are shown in Figure 4.2.

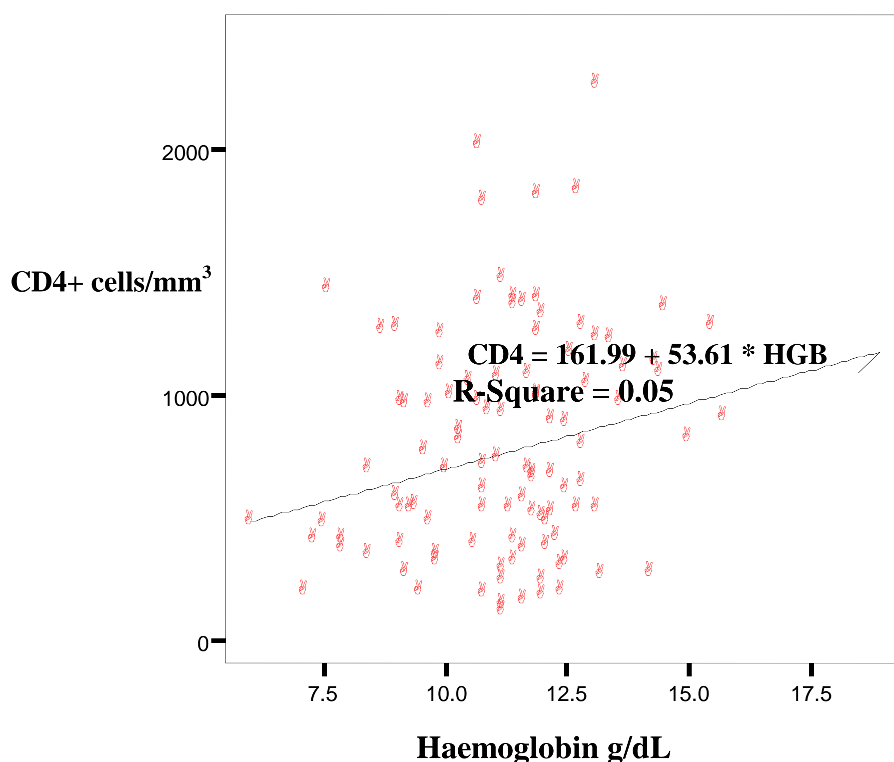

Figure 4.2: Relationship between CD4+ cells and haemoglobin

The correlations show that, PLWHA with suppressed immune system as indicated by low CD4+cells, are likely to have low levels of MCH, HGB, MCHC and higher levels of RDW, which are indicators of anaemia. The PLWHA with low CD4+ and high CD8+ cells, therefore, may need nutritional intervention with iron, folic acid, vitamin A, B_{12} and C as key ingredients required in haemoglobin synthesis (UNCF/UNU/WHO, 2001). However, low iron status or anaemia among PLWHA could be a defence strategy of the immune system to keep iron away from pathogens or as a response to the up regulation of TNF-α and IF-γ induced by increasing HIV viral load (Sarcletti *et al*., 2003).

High incidences of herpes zooster and Karposis Sarcoma were associated with high levels of RBC ($r = 0.212$, $p = 0.017$ and $r = 0.242$, $p = 0.01$, respectively) and HCT ($r = 0.184$, $p = 0.039$ and $r = 0.177$, $p = 0.046$, respectively), indicating that PLWHA with high iron status are at risk of herpes zooster and Karposis Sarcoma. This observation is supported by results of an *in vitro* study, which showed that iron is a co-factor in the development of Kaposis Sarcoma (Simonert *et al*., 1998). Iron is required for effective function of natural killer cells and is a co-factor of many enzymes involved in immune functions, such as iron-based myeloperoxidase, neutrophils, catalase and cytochromes (Staal *et al.,* 1993; Chester and Arthur, 1988). However, there is need for balanced intake of iron, as high intake may lead to increased oxidative stress, viral replication and bacterial infections (Delanghe *et al.,* 1998; Masawe *et al*., 1974). Therefore, the subjects with herpes zooster and Karposis Sarcoma infections need to take diet with low levels of iron.

4.4 Level of serum zinc

Level of serum zinc was analysed from 120 subjects and results presented in Table 4.12.

Table 4.12: The frequency and percentage of subjects in different categories of serum zinc levels

Category of serum zinc level µg/L	Male		Female		All subjects	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
< 70 µg/L	0	0	5	4.8	5	4.2
70-120 µg/L	0	0	11	10.4	11	9.1
> 120 µg/L	15	100	89	84.8	104	86.7

No male subjects (0 %) had below normal (< 70 µg/L) and normal serum zinc levels (70-120 µg/L), while 4.8 % and 10.4 % of the female subjects had below normal (< 70 µg/L) and normal serum zinc levels (70 to 120 µg/L), respectively. Majority of the female subjects (84.8 %) and all male subjects (100 %) had above normal serum zinc levels (> 120 µg/L). The mean serum zinc level for the male subjects (354.7 µg/L) was significantly (p = 0.006) higher than the levels for the female subjects (279.7 µg/L). Similar observation was reported by Vegas-Crespo *et al*. (2000), who attributed it to the difference in hormonal status between the male and the female subjects. The study indicates lower prevalence rate of zinc deficiency (13.3%) than 61 to 73% reported by Mburu *et al*. (2010) among similar study subjects living in Nakuru, Naivasha and Nairobi. However, Muriuki (2009) reported lower level of prevalence rate of zinc deficiency (1.5%) among similar study subjects living in Thika District. The difference in the prevalence rate of deficiency among PLWHA could be due to different food preparation practises and diet composition that influence nutrients bioavailability and concentration in food (Walingo, 2009).

4.4.1 Relationship between serum zinc and immunological status

Relationship between serum zinc level and immunological status of the subjects was determined using correlation coefficient and results presented in Table 4.13

Table 4.13: The relationship between serum zinc level and immunological status

Immunological indicator	Pearson Correlation co-efficient (r)	p = value
CD4+	-0.195	0.055
CD8+	-0.059	0.566
CD4/CD8+	-0.218	0.031

Low levels of all the immunological indicators (CD4+, CD8+ and CD4/CD8+) were associated with high levels of serum zinc, but only CD4/CD8+ cell ratio was significantly associated with high level of serum zinc ($r = -0.214$, $p = 0.031$). Although zinc strengthens cell mediated immune system, high levels could depress immunological system. This is because zinc induces activation of HIV-Tat and HIV-nucleocapsid proteins, which are involved in replication of HIV (Mocchegiani and Muzzioli, 2000). The HIV depletes CD4+ cells by suppressing their DNA repair mechanism, induces CD4+ cells synctia formation and other genes to initiate their own death. The immunological system responds by producing more CD8+ to reduce viral load, which results in low CD4/CD8+ cells ratio (Stine, 2005).

4.4.2 Relationship between serum zinc and clinical status

Relationship between serum zinc and clinical status of the subjects were determined by correlation co efficient and results presented in Table 4.14.

Table 4.14: The relationship between serum zinc level and some clinical conditions

Clinical condition	Correlation co-efficient	p=value
Pallor	-0.070	0.045
URTI	-0.087	0.347
Skin rash	-0.166	0.071
Diarhhoea	-0.017	0.852
Cough	-0.018	0.846
Fatigue	-0.095	0.320
Pneumonia	-0.002	0.984
Boils	-0.074	0.420
T.B	-0.057	0.538
Genital lesions	-0.175	0.056
Number of times treated for malaria	-0.068	0.534
Headache	-0.180	0.047
Fever	-0.195	0.035
Loss of appetite	-0.245	0.007
Lymphademopathy	0.04	0.666
Oedema	0.104	0.258
Herpes zooster	0.062	0.503
Karposis sarcoma	0.074	0.426
Weight loss	0.417	0.614

The prevalence of several clinical conditions were associated with low serum zinc levels: pallor (r = -0.007, p = 0.0453), URTI (r = -0.087, p = 0.347), skin rash (r = -0.166, p = 0.071), diarrhoea (r = -0.017, p = 0.852), cough (r = -0.018, p = 0.846), fatigue (r = -0.095, p = 0.02), pneumonia (r = -0.002, p = 0.984), boils (r = -0.074, p = 0.420), T.B (r = -0.057, p = 0.538), genital lesions (r = -0.175, p = 0.056) and number of times treated for malaria (r = -0.068, p = 0.534). However, only three clinical conditions were significantly associated with low serum zinc levels: headache (r = -0.18, p = 0.047), fever (r = -0.195, p = 0.035) and loss of appetite (r = -0.245, p = 0.007).

The findings of this study confirmed some documented clinical characteristics of zinc deficiency such as diarrhoea, skin lesions and loss of appetite (WHO/FAO, 2004). Most opportunistic infections were associated with low serum zinc because, zinc strengthens immune system. Zinc deficiency is accompanied by imbalance between Th1 and Th2 cells, decreased serum thymulin activity, decreased percentage of NK cell lytic activity and CD8+ cell activity thus, making the body vulnerable to opportunistic infections (Prasad, 2007). However, low serum zinc level is also caused by inflammation due to HIV or opportunistic infections, other than zinc deficiency (Mburu *et al*., 2010).

The prevalence of other clinical conditions was associated with high levels of serum zinc: lymphademopathy (r = 0.04, p = 0.666), oedema (r = 0.104, p = 0.258), herpes zooster (r = 0.062, p = 0.503), Karposis sarcoma (r = 0.074, p = 0.426) and weight loss (r = 0.417, p = 0.614). These findings also confirmed that zinc overload may be associated with increased activity of HIV and depressed immunity as reported by Tang *et al*. (1996). Therefore, a balanced intake of zinc is necessary for PLWHA because, both zinc deficiency and overload increase the rate of progression of HIV and AIDS.

4.5 Levels of trace elements in indigenous foods used in Butula division

A total of twenty four foods commonly used in Butula Division were analysed for levels of zinc, chromium, iron and selenium. The results are presented and discussed in the following sections.

4.5.1 Method validation

Reliability of the analytical method was checked by determination of linearity, recovery, detection limit and reproducibility. The results are presented and discussed below.

4.5.1.1 Detection limit, percentage recovery and precision of the method

A triplicate of pumpkin seeds sample and pumpkin seeds sample spiked with the trace elements were analysed for levels of the elements. The results were used to determine percentage recovery of the trace elements according to the following equation:

$$\text{Percentage recovery} = \frac{C_x}{C_o + C_s} \times 100$$ **Equation 4.1**

Where, $\mathbf{C_x}$ is level of the trace elements in spiked pumpkin seeds samples, $\mathbf{C_o}$ is level of the trace elements in pumpkin seeds samples and $\mathbf{C_s}$ is level of the trace elements spiked.

Results from repeated determinations (6 times) of the trace elements in pumpkin seeds were used to determine random error of analysis using coefficient of variation (CV), which was calculated using the following equation:

$$\text{Coefficient of variation} = \frac{s}{\bar{x}} \times 100$$ **Equation 4.2**

Where, **s** is absolute standard deviation and $\bar{x}$ is mean level of the trace elements.

Detection limit was defined as three times standard deviation of the blank value; whereby y-intercept was taken as satisfactory estimate of the blank and statistics $\mathbf{S_{xy}}$ as estimate of

standard deviation. Table 4.15 gives a summary of percentage recovery, detection limit and precision of the elements.

Table 4.15: The detection limit, percentage recovery and precision of the analytical method

Elements	Detection limit (ppm)	Recovery %	Precision (CV)
Zinc	0.07	98	2.0
Iron	0.3	91	1.3
Chromium	0.005	94	1.5

Recoveries of the elements in the method used were in the range of 91 % to 98 %, with the highest level recorded in zinc and the lowest in iron. Recovery rate is usually reduced by element loss due to volatilization and adsorption on the walls of glassware (Kumpulainen *et al.*, 1979). Level of precision was high in iron (1.3%) and lowest (2 %) in zinc. Low precision observed in zinc (2 %) could be due to flame instability or high levels of silicon in the plant materials such as grains that interfere with zinc absorption, especially at low zinc levels (Lujen, 1992). Furthermore, concentration error of 1 to 2 % is common in spectrophotometers and photometers due to indeterminate uncertainty of 0.3 % of transmittance caused by limited readout resolution, Johnson noise and dark current (Skoog and Leary, 1992).

The lowest detection limit was observed in chromium (0.005 ppm) that was similar to that indicated by the manufacture (0.005 ppm). On contrary, higher detection limit was observed in iron (0.315 ppm) and zinc (0.0712 ppm) that are above the ones indicated by the manufacturer (0.005 ppm and 0.002 ppm, respectively). Rise in the detection limit of iron and zinc could be due to flicker noise resulting from the long term drift in direct current

amplifiers, meters and galvanometers (Skoog and Leary, 1992). Therefore, periodic calibration of analytical instrument is necessary to check against change in the detection limit. The recovery rate, precision levels and detection limits were generally satisfactory in all the elements. This method is, therefore, suitable for analyzing levels of the trace elements in food.

4.5.1.2 Linearity

The linear range of AAS used in the analysis was checked by regression equation of the calibration curve of the standards. Concentrations of the standards used bracketed analyte concentrations in the food samples. The calibration curves (Appendices 12 to 14) were obtained by analysis of a series of analyte concentrations (ppm) corresponding to 0.5 to 2 ppm for chromium; 2 to 10 ppm for iron and 1 to 3 ppm for zinc. The curves obtained were subjected to linear regression analysis: $y = mx + c$, where; y = absorbance, x = concentration of analyte (ppm), r = correlation of co-efficient. Systematic error caused by contamination from sample preparation procedure and spectral background was reduced by subtracting blank values from sample readings. Table 4.16 gives regression equations and Pearson correlation co-efficient of calibration curves used.

Table 4.16: The regression equations and Pearson correlation co-efficient of calibration curves for zinc, iron and chromium

Parameter	Zinc	Iron	Chromium
Regression Equation	$y = 0.158x + 0.040$	$y = 0.039x + 0.005$	$y = 0.083x + 0$
Correlation Coefficient, r	0.976	0.998	0.999

The highest correlation was observed in chromium curve (0.999), while the lowest (0.976) was in zinc. The correlation coefficients were slightly lower than ideal value of 1, due to spectral and chemical interferences that reduce linearity of calibration curve (Lujen, 1992). However, the correlation co-efficient values obtained when compared with calibration curves drawn (Appendices 7 to 9), show good linearity of the analytical method used. Therefore, over 95% variability in the calibration curves could be accounted for by levels of zinc (R^2 = 0.953), iron (R^2 = 0.996) and chromium (R^2 = 0.998) in the analyte.

The regression equations observed for zinc, iron and iron were: $y = 0.158x + 0.040$, $y = 0.039x + 0.005$ and $y = 0.083x + 0$, respectively. The y - slope intercepts for regression equations for zinc (0.040) and iron (0.005) were slightly above origin point of the graph (0,0), whereas chromium y - slope intercepted the origin point of the graph (0,0). The high slope intercepts of zinc and iron could be due to flicker or environmental noise picked by the instrument during analysis and converted to electrical signal, which was interpreted by the transducer as analyte. Flicker noise may be reduced by periodic calibration of the instrument, whereas environmental noise could be reduced by shifting analyte signal to frequencies with low noise interference (Skoog and Leary, 1992).

The analytical method used showed good linearity within the concentration range of the analytes. Therefore, this method is suitable for analyzing levels of the trace elements in the food.

4.5.2 Levels of the trace elements in cereal grains and tuber roots

Cereals analysed included: four types of sorghum and two types of finger millet, whereas tubers included: cassava tubers and three species of sweet potatoes. Levels of zinc, iron,

chromium and selenium are presented in Table 4.17 as mean levels of the elements in 100 g dry mass of the food samples ± standard deviation.

Table 4.17: The mean levels of zinc, chromium, iron and selenium in cereal grains and tuber roots

Cereal grains and tuber roots			Levels (mg / 100 g); n = 3			
English name	**Botanical name**	**Local name**	**Iron**	**Zinc**	**Chromium**	**Selenium**
Cereals			$\bar{x}$±sd	$\bar{x}$±sd	$\bar{x}$±sd	$\bar{x}$±sd
Sorghum	*Sorghum bicolor Sp.*	Werenje	7.50±0.6	1.60±0.01	0.28±0.005	BDL
Sorghum	*Sorghum bicolor Sp.*	Sabina	11.50±0.7	1.70±0.2	0.28±0.005	0.078±0.01
Sorghum	*Sorghum bicolor Sp.*	Ayeye	19.50±0.5	1.60±0.2	0.33±0.004	0.007±0.001
Sorghum	*Sorghum bicolor Sp.*	Nyir matin	8.90±0.3	1.35±0.03	0.30±0.01	0.043±0.002
Finger Millet (black)	*Eleusine Coracana Sp.*	Bule	9.13±0.06	1.70±0.2	0.65±0.04	0.043±0.002
Finger Millet (brown)	*Eleusine Coracana Sp.*	Bule	7.95±0.7	1.30±0.07	0.20±0.02	BDL
Tuber roots						
Cassava tubers	*Manihot esculenta Sp.*	Mioko	6.00±0.8	0.44±0.01	0.13±0.01	0.004±0.001
Sweet potatoes (white cover white flesh)	*s. tubirosam Sp.*	Mabwoni ile Sirimi	12±0.8	0.65±0.02	0.25±0.03	0.032±0.001
Sweet potatoes (brown cover brown flesh)	*s. tubirosam Sp.*	Mabwoni ile Sirimi	6.5±0.2	0.58±0.04	0.13±0.01	0.03±0.002
Sweet potatoes (white cover brown flesh)	*s. tubirosam Sp.*	Mabwoni ile Sirimi	9.8±0.5	0.55±0.02	0.25±0.08	0.05±0.005

The highest level of iron (19.5 mg/100g) was observed in a sorghum species known locally as *Ayeye*, whereas the lowest level (6.0 mg/100g) was recorded in cassava tubers. The average level of iron in the sorghum species was 11.85 mg/100g, with the highest level (19.5 mg/100g) in *Ayeye* and lowest level (7.5 mg/100g) in *Werenje.* Tuber roots had slightly lower

levels of iron (mean 9.4 mg/100g), with the lowest level (6.0 mg/100g) in cassava tubers and the highest level in sweet potato species with white cover and white flesh (12.0 mg/100g).

Levels of iron observed in the tubers were close to those reported in Indian potatoes (6.97 mg/100g) by Singh and Garg (2006), but higher than those reported in Finnish potatoes, 3.4 mg/100g (Ekholm *et al.,* 2007). The mean level of iron in finger millet was 8.54 mg/100g, which ranged from 9.13 mg/100g in species with black grains to 7.95 mg/100g in species with brown grains. Levels of iron in the cereals (ranging from 7.5 to 9.13 mg/100g) are below values (17.8 to 19.4 mg/100g) reported in Indian cereals (Singh and Grag, 2006), but higher than levels (3.2 to 5.1 mg/100g) reported in cereals from Tanzania (Towo *et al*., 2006).

The highest level of zinc (1.7 mg/100g) was observed in both sorghum (*Sabina*) and finger millet with black grains, whereas the lowest level (0.13 mg/100g) was recorded in both cassava and sweet potato tubers with brown cover and brown flesh. On average, cereals contained higher levels of zinc (mean 1.54 mg/100g) than tubers (mean 0.56 mg/100g). The levels in the cereals analysed are slightly below those reported by Muchemi *et al*. (2007) in cereals from Githurai market, Thika District, which had: sorghum (2.1 mg/100g) and finger millet (2.18 mg/100g) and Muriuki (2009) in Bulrush millet from Meru district (3.8 mg/100g). Zinc levels of zinc reported in other countries are close to those observed in the grains analysed, such as 1.73 mg/100g in finger millet and 2.47 mg/100g in sorghum in India (Hemalanthan *et al*., 2007; Singh and Garg, 2006) and 1.6 to 2.3 mg/100g reported in cereals from Tanzania (Towo *et al*., 2006).

Levels of chromium in the cereals (ranging from 0.20 to 0.65 mg/100g) were close to those reported by Muchemi *et al*. (2007) in cereals from Githurai market, Thika District (ranging

from 0.28 to 0.59 mg/100g). The levels in the tubers (ranging from 0.25 to 0.13 mg/100g) were close to that reported in potatoes in India (0.164 mg/100g), but the levels in the cereals (ranging from 0.2 to 0.65 mg/100g) were higher than those in Indian cereals (0.188 mg/100g) (Singh and Garg, 2006).

Selenium was below the detection limit (BDL) in finger millet (brown) and sorghum (*Werenje*), while the highest level was observed in another species of sorghum locally known as *Sabina* (0.078 mg/100g). The levels observed in finger millet species (BDL to 0.043) were slightly above 0.0198 mg/100g in finger millet from Meru District (Muriuki (2009) and cereals from Githurai market, Thika District (ranging from 0.0198 to 0.0091 mg/100g) (Muchemi *et al*., 2007). Selenium levels in the tubers were close to those reported in Indian potatoes (0.0325 mg/100g) (Singh and Garg, 2006), but lower than those observed in Finnish potatoes (0.003 mg/100g) (Ekholm *et al.,* 2007).

4.5.3 Levels of the trace elements in nuts, vegetable and pulses seeds

The nuts seeds included: sesame seeds, ground and bambara nuts; the vegetable seeds included: spider herb, sunn hemp, bush okre (*jute*) and two species each of pumpkin and amaranthus seeds, and the pulses included: soya beans, cow peas and two species of green grams. These foods were analysed for levels of zinc, iron, chromium and selenium and the results are presented in Table 4.18 as mean levels of the elements in 100 g dry mass of the food samples ± standard deviation.

Table 4.18 The mean levels of iron, chromium, zinc and selenium in vegetable, nuts and pulses seeds

Vegetables, nuts and pulses seeds			**Levels of elements (mg/100g) n = 3**			
English name	**Botanical name**	**Local name**	**Iron**	**Zinc**	**Chromium**	**Selenium**
Vegetables			$\bar{x}$±sd	$\bar{x}$±sd	$\bar{x}$±sd	$\bar{x}$±sd
Pumpkin seeds (smooth-brown)	*Cucurbita pepo Sp.*	Liondo	5.10±0.8	4.60±0.5	0.300±0.00	0.014±0.005
Pumpkin seeds (hairy - smooth)	*Cucurbita pepo Sp.*	Liondo	7.00±0.8	4.50±0.1	0.25±00	0.063±0.004
Spider herb	*Gynanadropsis gynandra Sp.*	Tsifwa tsia Saka	19.40±0.13	3.88±0.07	0.30±0.008	0.068±0.004
Amaranths seeds (brown)	*Amaranths hybridus Sp.*	Tsifwa tsia Lidodo	11.70±1.0	3.20±0.3	0.20±0.08	0.087±0.005
Amaranths seeds (black)	*Amaranths hybridus Sp.*	Tsifwa tsia Lidodo	6.70± 0.8	2.25±0.02	0.26±0.002	0.070±.001
Sunn hemp	*Crotalaria brevidens Sp.*	Tsifwa tsia Miro	3.50±0.8	2.95±0.08	0.13±0.094	0.061±0.002
Bush okre (jute)	*Corchorus olitorius Sp.*	Tsifwa tsia Mrere	12.30±0.0	2.81±0.08	0.13±0.008	0.024±0.002
Nuts						
Groundnuts	*Arachis hypogaea Sp.*	Tsinjugu	7.75±0.07	2.77±0.06	0.28±0.04	0.018±0.007
Sesame seeds	*Sesame indicum Sp.*	Tsifwa tsia tsinuni	17.10±0.2	4.13±0.08	0.10±0.01	0.028±0.001
Bambara nuts	*Vigna subterranea Sp.*	Tsifwa tsia Bambara	5.30±0.7	1.51±0.05	0.13±0.005	BDL
Pulses						
Cow peas	*Vigna catjang Sp.*	Tsingo'li tsia Likhubi	11.0±0.5	2.58±0.004	BDL	0.042±0.003
Soya beans	*Glycine Max Sp.*	Tsingo'li tsia Soya	9.85±1.0	3.85±0.05	BDL	0.016±0.0
Green grams	*Phaseolus aureus Sp.*	Tsingo'li tsia Dengu	7.98±0.4	1.91±0.03	0.75±0.005	0.028±0.004
Green gram	*Phaseolus aureus Sp.*	Tsingo'li tsia Olayo	5.73±0.04	2.13±0.02	BDL	0.096±0.004

Levels of iron were ranging from 3.5 mg/100g in sunn hemp seeds (*Miro*) to 19.4 mg/100g spider herb seeds (*Saka*). In the pulses, iron levels ranged from 5.73 to 11.0 mg/100g, with

the highest level in cow peas and the lowest level in green grams species, locally known as *Olayo*. Iron levels in the pulses were higher than those reported in Indian pulses (ranging from 4.97 to 4.55 mg/100g) (Singh and Grag, 2006). Levels of iron observed in the nuts were slightly higher than those reported in USA peanut (4.58 mg/100g) and sesame seeds (14.55 mg/100g) (USDA, www.nal.usda.gov, 1996).

Zinc levels observed in the nuts, pulses and vegetable seeds ranged from 1.51 to 4.6 mg/100g, with the highest level in pumpkin seeds (smooth-brown) and the lowest level in cassava tubers. Levels of zinc observed in the pulses (ranging from 1.91 to 3.85 mg/100g) were close to what was reported in pulses (2.94 mg/100g) from Githurai market, Thika District (Muriuki, 2009). Also the zinc levels in the nuts (ranging from 1.51 to 4.13 mg/100g), were close to those reported in nuts in other countries, such as 2.6 mg/100g in Sweden (Rodushkin *et al.*, 2008) and 2.5 to 6.9 mg/100g in Spain (Cabrera *et al*., 2003). On average, vegetable seeds had the highest levels of zinc (3.5 mg/100g) that ranged from 2.25 mg/100g in amaranths seeds (black) to 4.6 mg/100g in pumpkin seeds (smooth brown). Zinc levels observed in pumpkin seeds (ranging from 4.5 to 4.6 mg/100g) are slightly lower than those reported in pumpkin seeds (5.2 mg/100g) from Githurai market, Thika District (Muriuki, 2009).

The highest level of chromium (0.75 mg/100g) was recorded in green grams (*Dengu*), whereas it was below detection limit (BDL) in another species of green grams (*Olayo*), cow peas and soya beans. Levels of chromium observed in the nuts (ranging from 0.1 to 0.28 mg/100g) are close to those reported by Cabrera *et al.* (2003) in Spain nuts (ranging from 0.1 to 0.25 mg/100g). On the average, vegetable seeds had the highest level of chromium that

ranged from 0.13 mg/100g in sunn hemp and bush okre to 0.3 mg/100g in pumpkin seeds (smooth brown) and spider herb.

The highest level of selenium (0.028 mg/100g) was observed in green grams (Olayo), whereas selenium was below the detection limit (BDL) in bambara nuts. Levels of selenium in the nuts were similar to those reported in nuts in other countries, such as Sweden 0.06 mg/100g (Rodushkin *et al.*, 2008) and Mauritius 0.0048 to 0.0192 mg/100g (Subratty *et al*., 2004). Levels of selenium in the pulses (ranging from 0.096 to 0.016 mg/100g) were similar to those reported by Subratty *et al*., (2004) in Mauritian pulses (ranging from 0.093 to 0.001 mg/100g), but were slightly higher than those reported in pulses (0.0013 mg/100g) from Githurai market, Thika District (Muriuki, 2009) and in pulses from Finland (0.003 mg/100g) (Ekholm *et al*., 2007).

4.5.6 Mean levels of trace elements in the major food groups

A summary of mean levels of the elements in 100 g dry mass of the major food groups analysed are presented in Table 4.19.

Table 4.19: The range and mean levels of the trace elements in the major food groups

Foods	Levels of trace elements (mg/100g)							
	Iron $\bar{x}$±sd	Range	Zinc $\bar{x}$±sd	Range	Chromium $\bar{x}$±sd	Range	Selenium $\bar{x}$±sd	Range
Cereal grains	10.7±4.5	7.5-19.5	1.5±0.2	1.3-1.7	0.30±0.2	0.2-0.6	0.03±0.03	BDL-0.08
Pulses	8.6±2.3	5.7-11.0	2.7±0.9	1.9-3.9	0.20±0.4	BDL-0.7	0.05±0.04	0.02-0.09
Vegetable seeds	9.4±5.5	3.5-19.5	3.5±0.9	2.3-4.6	0.22±0.07	0.1-0.3	0.05±.003	0.01-0.08
Nuts	10.0±6.2	5.3-17.1	2.8±1.3	1.5-4.1	0.17±0.1	0.1-0.3	0.02±0.01	BDL-0.03
Tubers roots	6.8±2.0	4.8-9.8	0.6±0.1	0.4-0.7	0.19±0.07	0.1-0.3	0.02±0.01	0.01-0.03
Mean	9.3±4.3	6.8-10.7	2.3±1.3	0.6-3.5	0.23±0.18	0.17-0.3	0.04±0.03	BDL-0.09

The highest mean levels of iron (10.7 mg/100g) and chromium (0.3 mg/100g) were recorded in cereals and were in the range of 7.5 to 19.5 mg/100g and 0.2 to 0.6 g/100g, respectively. Respective selenium and zinc levels in cereals were modest (0.03 and 1.5 gm/100g) and were ranging from 1.3 to 1.7 gm/100g and BDL to 0.08 mg/ 100g respectively. Pulses had modest respective levels of iron (8.6 mg/100g), zinc (2.7 mg/100g) and chromium (0.2 mg/100g) that ranged from 5.7 to 11.0 mg/100g, 1.9 to 3.9 mg/100g and BDL to 0.7 mg/100g. The highest level of selenium was observed in pulses (0.05 mg/100g) and vegetable seeds (0.5 mg/100g), which ranged from 0.02 to 0.09/100g and 0.01 to 0.08/100g, respectively.

Vegetable seeds had the highest level of zinc (3.5 mg/100) and modest levels of iron (9.4 mg/100g) and chromium (0.22 mg/100g). Levels of iron, zinc and chromium in vegetable seeds ranged from 3.5 to 19.4 mg/100g, 2.3 to 4.6 mg/100g and 0.1 to 0.3 mg/100g, respectively. Nuts had the lowest level of selenium (0.02 mg/100g) and chromium (0.17 mg/100g), which ranged from BDL to 0.03 mg/100g and 0.13 to 0.3 mg/100g, respectively. Levels of zinc (2.8 mg/100g) and iron (10.0 mg/100g) in nuts were modest and ranged from 1.5 to 4.1 mg/100g and 5.3 to 17.1 mg/100g respectively. The lowest levels of iron and zinc were observed in tubers and were ranging from 4.8 to 9.8 mg/100g and 0.4 to 0.7 mg/100g respectively. However, respective chromium (0.19 mg/100g) and selenium (0.02 mg/100g) levels in tuber roots were modest and ranged from 0.1 to 0.3 mg/100g and 0.03 to 0.03 mg/100g.

The overall mean contents of the elements in the major food groups were in the range of 10.7 to 6.8 mg/100g for iron, 3.5 to 0.56 mg/100g for zinc, 0.34 to 0.17 mg/100g for chromium and 0.05 to 0.015 mg/100g for selenium. Highest level of zinc 3.5 mg/100g and selenium 0.055 mg/100g were observed in vegetable seeds, whereas cereals contained highest levels of chromium (0.3 mg/100g) and iron (10.7 mg/100g). There was negative significant correlation between iron/zinc ratio and zinc ($r = -0.606$, $p = 0.002$), indicating possible negative interaction between iron and zinc during their uptake by the plants.

The study indicates that, cereals which are widely consumed (35 %) in Butula Division contain high levels of iron (10.7 mg/100g), chromium (0.3 mg/100g) and selenium (0.03 mg/100g), but low levels of zinc (1.5 gm/100g), as compared to vegetable seeds (3.5 mg/100g) and pulses (2.8 mg/100) that are least consumed (22 % and 4 %, respectively). If on average, one person consumes 50 g of the cereals per meal (GOK, 2006), then, for two

meals a day, on average, the cereals would provide 0.3 mg of chromium, 0.03 mg of selenium, 10.7 mg of iron and 1.5 mg of zinc. The levels of iron and zinc in the cereals can meet 39.6 and 16 % iron RDA and 18.2 and 23.4 % zinc RDA for adult male and female, respectively in low iron (5 %) and zinc (15 %) bioavailability diet (FAO/WHO, 1998).

In order to meet iron and zinc RDA, the subjects need to take a large amount of the cereals per meal or take iron and zinc supplements or increase proportion of nuts and vegetable seeds, which have higher levels of the elements. The low amount of iron and zinc in the cereals analysed can also meet RDA, if meat is introduced in the diet to improve iron and zinc bioavailability. This is because, meat contains the sulfhydryl groups of amino acids especially, cysteine that reduces less soluble iron (III) to more soluble iron (II) or form soluble iron-peptide complex in the intestine (Cámara *et al*., 2005).

4.6 Bioavailability of the trace elements in selected indigenous foods from Butula division

4.6.1 Development of algorithm for absorption of selenium

Data on selenium absorption was obtained from the internet after carrying out a search using key words, such as selenium intervention, selenium absorption and selenium retention. Table 4.20 gives a summary of selenium absorption data obtained from the internet.

Table 4.20: The Sources of data, intervention sample size, duration of study, pre-study serum selenium levels, percentage of selenium absorbed and retained

Source of data	Intervention	Sample Size (N)	Duration of study (days)	Pre-study serum Selenium (μg/L)	Selenium absorbed (%)	Selenium retention (%)
Bügel *et al.* (2008)	Supplement of Se-yeast (300 μg Se/day) for 10 weeks. Absorption estimated using a single dose of Se-yeast intrinsically labelled with ^{77}Se (stable isotope).	12	1	182	89	74
Torre *et al.* (1991)	Supplement of Se-enriched bread and Se-rich meat . 5 weeks of low Se intake followed by 9 weeks of either low supplement (55 μg Se/day) or high supplement (215 μg Se/day), absorption estimated using a single dose of Se-yeast intrinsically labelled with ^{77}Se (stable isotope).	23	35-98	65-138	11-80	-74-16
Hawkes *et al*. (2003)	Supplement of long grain white rice and beef. 3 weeks of low Se intake (47 μg/day) followed by 99 days of either low Se supplement (14 μg Se/day) or high supplement (297 μg Se/day), absorption estimated with a single dose of Se-yeast intrinsically with ^{77}Se (stable isotope). Se-rich meat 47 μg/day for 21 days on 22 day diet changed into 14 μg or 297 μg of Se/day for the test meal	11	3-98	107-122	12-88	12-88
King (2001)	Supplement of eggs intrinsically labelled with ^{76}Se (stable isotope). Dietary selenium was 150 μg /day for non expecting women	6	21	130	81	11
Finley (1999)	Supplement of broccoli intrinsically labelled with ^{82}Se (stable isotope). Low selenium diet (32.6 μg /day) or high selenium diet (226.5 μg /day)	27	86	Not given	66.4-74.4	58.5-59.8
Fox *et al.* (2004)	Supplement of salted and cooked fish and yeast intrinsically labelled with ^{74}Se and ^{77}Se, respectively (stable isotope). Dietary selenium in yeast, salted and cooked fish group were 115.5, 128.7, and 151.3 (μg /day), respectively	35	3	Not given	53.5-90.4	59.3-86.2

Selenium absorption ranged from 11 to 90.4 %, whereas pre-study serum selenium ranged from 65 to 182 μg/L. The duration of intake of the test meals ranged from one day to 98 days. In studies done by Torre *et al.* (1991) and Hawkes *et al.* (2003), the subjects test meals changed from either high to low selenium meals or low to high selenium meals. Sources of

selenium in the test meals included yeast, rice, eggs, fish, bread and meat. The meals were intrinsically labelled with ^{74}Se, ^{77}Se and ^{82}Se stable isotopes. The percentage of selenium absorbed (%) was regressed against the duration of intake of the supplement (days), the dietary level of selenium (µg) and the pre-study serum level of selenium (µg/dL). The results of regression analysis are presented in Table 4.21.

Table 4.21: The predictor variables of selenium absorption and coefficients

Predictor variables of selenium absorption	Coefficients B	p = value
Dietary level of selenium (µg)	0.148	0.000
Pre-study serum selenium (µg/L)	-0.047	0.615
Duration of intake of supplement (days)	-0.120	0.164
Constant	50.963	0.000

The dietary level of selenium (B = 0.148; $p < 0.000$) was the most influential positive significant predictor, whereas the pre-study serum level of selenium and the duration of intake of selenium supplement had negative corresponding co-efficient (B = -0.047 and B = -0.120) that were insignificant ($p = 0.615$ and $p = 0.164$, respectively). The predictors accounted for over a half of the variance in absorption of selenium (adjusted $R^2 = 0.533$), which was highly significant at $p < 0.000$. For every increase in the dietary level of selenium by 0.148 units, the amount of absorbed selenium increases by one (1) unit. Therefore, the regression equation for absorption of selenium is $r = 0.148x + 50.963$, where x is the total level of dietary selenium (µg/day) and r is the percentage of absorbed selenium (%) in the food.

The constant (50.963) shows that absorption of selenium is partly independent of the dietary and serum selenium levels and duration of selenium intake, but is influenced by other factors,

possibly by the active transport. The constant (50.963) may be due to the effect of selenomethionine that makes up about 50 % of total selenium in food and is highly absorbed (over 90 %), mainly by Na^{+}-dependent carrier mediated process (SOPFANS, 2009). The co-efficient of the dietary level of selenium (0.148) indicates that absorption of selenium partly depends on the concentration and therefore, is partly passive (diffusion dependant). Inorganic forms of selenium in foods, such as selenate and selenite are not absorbed against concentration gradient, but are absorbed by diffusion and could be attributed to the slope (0.148) observed in the model (McConnell and Cho, 1965).

Although little data were used to develop the model, plots of residual squares (Figures 4.3 and 4.4) obtained from regression analysis of selenium absorption show that, assumptions about linearity, normal distribution and equal variances were met.

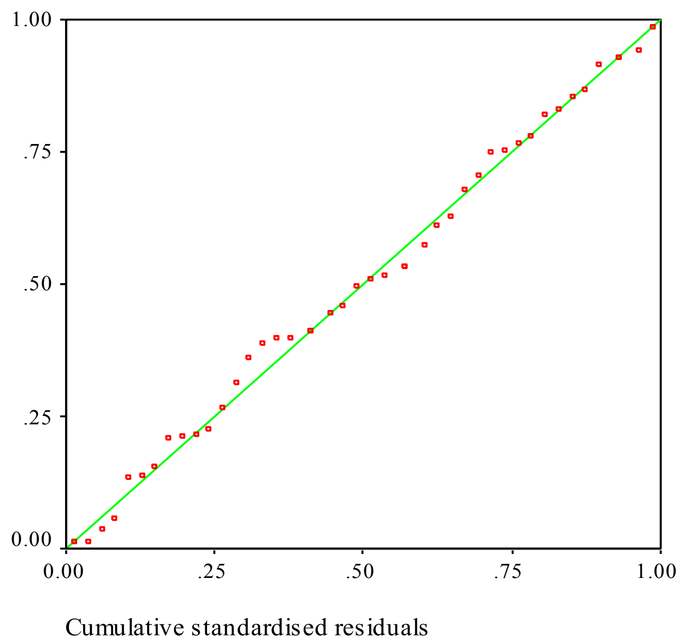

Figure 4.3 Probability plots of residuals

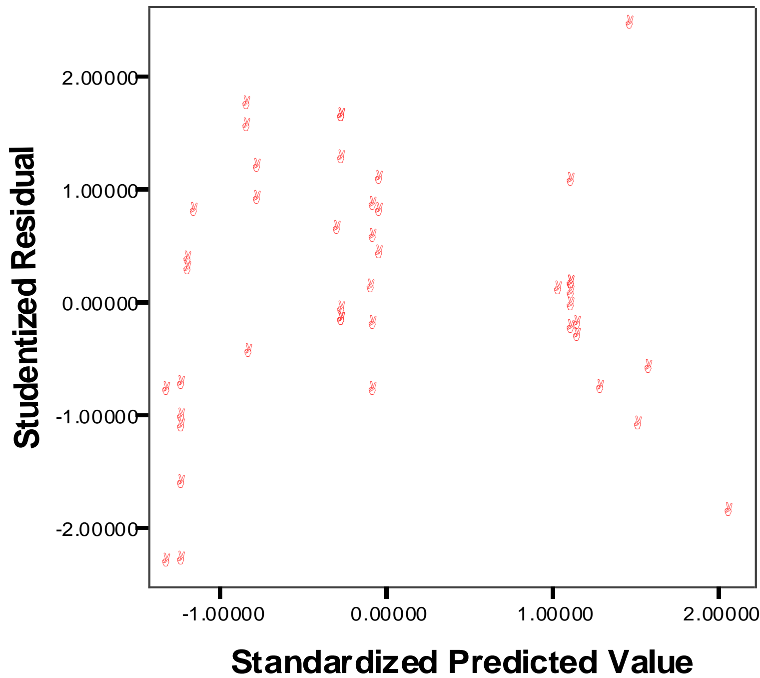

Figure 4.4: A probability plot of studentized residuals against standardized predicted values

The model was used to estimate selenium absorption in millet, sesame and pumpkin seeds and results are presented in Table 4.22.

Table 4.22: The total amount of selenium in and absorbed from millet, pumpkin and sesame seeds

Selenium	Sesame seeds 100 g	Millet grains 100 g	Pumpkin seeds 100 g
Total level (µg)	28.0	43.0	63.0
Bioavailable (%)	55.1	57.3	60.3

The highest percentage of bioavailable selenium was observed from pumpkin seeds (60.3 %) µg), followed by millet (57.3 %) and sesame seeds (55.1 %). Therefore, intake of 100 grams

of pumpkin seeds, millet grains and sesame seeds would deliver 38, 24.7 and 15.4 µg, respectively of bioavailable selenium. Although no data is available on the mount of selenium that must be absorbed in order to meet physiological needs of the body, it is estimated that intake of food that contains 27 µg/day selenium by a 65 kg man is adequate to maintain satisfactory levels of plasma selenium level (> 80 µmol/L) and glutathione peroxidise activity (> 0.3 mmol NADPH oxidised/min/L) (WHO/FAO, 2004). Therefore, intake of 100 g of any of the foods: millet grains, pumpkin or sesame seeds may meet the daily selenium requirements of the subjects.

4.6.2 Algorithm bioavailability studies of zinc and iron in the foods

Mathematical model for iron absorption developed by Hallberg and Hulthĕn (2000) was used to estimate percentage of bioavailable iron. The model is based on absorption of iron from a wheat roll (basal absorption 22.1%) without inhibitors or enhancers and adjusted to a reference dose absorption of 40.0 %, which corresponds to a serum ferritin concentration of 23 µg/L. The basal absorption is multiplied by factors which affect absorption and these include phytate, calcium and ascorbic acid as given in Equation 3.2. The algorithm was used to estimate bioavailable iron in millet grains, sesame and pumpkin seeds. These factors were computed using equations in Appendix 11.

Zinc bioavailability in the foods was estimated using equation 3.6. The equation describes a saturable, carrier-mediated trivariate model with two independent variables: TDZ and TDP, where: TDZ is total dietary zinc (millimoles) and TDP is total dietary phytate (millimoles) in food. The dependent variable is total absorbed zinc (TAZ) in millimoles, while the three parameters of the model include: maximum zinc absorbed (A_{max}), which is related to the number of zinc transport receptors, equilibrium dissociation constant of zinc-phytate complex

(K_P) and equilibrium dissociation constant of zinc receptor complex (K_R). The values of A_{max}, K_P and K_R are 0.11, 0.06 and 1.46, respectively and were obtained from estimates of Hunt *et al*. (2008) in a low zinc bioavailability diet.

Levels of bioavailable zinc and iron were estimated using the algorithms (Equations 3.5 and 3.6) in 100 g of millet grains, sesame and pumpkin seeds and results presented in Table 4.23.

Table 4.23: The levels and percentages of bioavailable iron and zinc estimated using the algorithms in 100 grams of millet grains, sesame and pumpkin seeds

Parameters		Sesame seeds 100 mg	Finger Millet grains 100 mg	Pumpkin seeds 100 mg
Calcium (mg)		637.8	262.1	60.9
Phytate (mg)		576.0	217.0	602.8
Ascorbic acid (mg)		0	0	1.9
Iron	Total (mg)	17.1	9.13	7.0
	Bioavailable (%)	1.34	1.79	1.32
	Bioavailable (mg)	0.80	0.01	0.25
Zinc	Total zinc (mg)	4.13	1.70	4.50
	Bioavailable (%)	9.77	32.73	11.19
	Bioavailable (mg)	0.404	0.56	0.50

Bioavailable iron in the foods ranged from 1.32 to 1.79 %, with the highest level in millet grains and the lowest level in pumpkin seeds. The highest percentage of bioavailable iron in millet (1.79 %), could be due to lower level of phytate (217.0 mg/100g). Pumpkin seeds had slightly lower level of bioavailable iron (1.32 %) than sesame seeds (1.34 %), because it has higher level of phytate (602.8 mg/100g) as compared to the levels in sesame seeds (576.0

mg/100g). Although ascorbic acid facilitates absorption of iron by forming soluble complex with iron, the effect of ascorbic acid on absorption of iron in pumpkin seeds was very low, due to its low level (1.9mg/100g) (Kies and McEndrea, 1982). Low level of bioavailable iron in sesame seeds could be due to high levels of calcium (637.8 mg) and phytate (576.0mg) because, calcium and iron compete for absorption site in the mucosal cells whereas, phytate forms insoluble complex with iron (Herland and Oberleas, 1987; Hallberg and Hulthĕn, 2000).

The highest bioavailable zinc (32.73 %) was observed in finger millet, while sesame seeds had the lowest bioavailable zinc (9.77 %). This could be because in sesame seeds, zinc had highest interaction with phytate (576.0 mg/100g) as compared to millet, which has low phytate level (217.0 mg/100 g), (Hurrel *et al.*, 1992). Phytate reduces absorption of zinc in a dose dependent manner because, it forms insoluble complex with Zn^{2+} (Fredlund *et al.*, 2006; Herland and Oberleas, 1987). Although calcium on its own does not affect absorption of zinc and was not factored in zinc absorption algorithm, it enhances inhibitory effect of phytate significantly when [calcium x phytate] : [zinc] molar ratio exceeds 200 (Yan *et al.*, 1996; Ellis *et al.*, 1987). This is in line with low zinc bioavailability (9.77 %) observed in the sesame seeds that had high levels of [calcium x phytate] : [zinc] of 217, but contradicts low zinc bioavailability (11.19 %) observed in pumpkin seeds which had low level of [calcium x phytate] : [zinc] of 20. Therefore, [calcium x phytate]: [zinc] ratio has limited predictive value of zinc bioavailability as also reported by Lönnerdal (2000).

The levels of bioavailable iron determined (1.32 to 1.79 %), were lower than estimates in maize (4.3 to 4.6 %) and beans (6.0 to 6.1 %) from Nigeria determined using a similar method. The difference could be explained by higher levels of phytate (217.0 to 602.8 mg) in

the grains analysed, compared to levels of phytate (289 to 446 mg) in maize and beans from Nigeria (Beiseigel *et al*., 2007). However, this study factored the inhibitory effects of calcium on absorption of iron, which had not been factored in the estimates reported by Beiseigel *et al*. (2007). Phytate forms insoluble chelates with iron resulting into depressed iron absorption, while calcium and iron compete for the absorption site (Herland and Oberleas, 1987; Hallberg and Hulthĕn, 2000). The models were used to identify foods for formulation and promotion.

4.6.3 In vitro nutrients bioavailability studies

Dialyzable zinc, iron and chromium were determined in millet grains, sesame and pumpkin seeds when prepared in formulation with the ratio of 4:4:1. A summary of bioavailability of the elements is presented in Table 4.24 as percentage of total levels of the elements in the food formulation.

Table 4.24: The total levels and percentage bioavailability of zinc, chromium and iron in the food supplement

Parameter	Iron	Zinc	Chromium
Total (mg)	26.40	3.20	0.23
Bioavailable (%)	24.20	34.40	17.40

Dialyzable iron, zinc and chromium in the foods were: 24.2 %, 34.4 % and 17.4 % respectively, which are equivalent to 6.4 mg of iron, 1.1 mg of zinc and 0.04 mg of chromium in the grains. The difference in dialyzability may be due to existence of different forms of the elements in food. Trivalent iron and chromium are known to be less soluble, because, as the pH increases when food moves from the acidic stomach to alkaline intestine, $Fe(H_2O)_6^{3+}$

undergoes hydrolytic polymerisation involving deprotonation to form hydroxo iron species $[Fe_x(OH)_y(H_2O)_n]^{(3x-y)+}$ and finally a less soluble $Fe(OH)_3$ is formed (Crichton, 1991; Garrow *et al*., 2000). The divalent iron and zinc, on the other hand are more soluble (da Silva and William, 1991).

Levels of dialyzable iron (24.2 %) observed in the grains are similar to those reported by Grewal and Jood (2006) in pulses (26.6 to 28.2 %), but higher than levels (4.13 to 8.05 %) reported in cereals from India (Hemalanthan *et al*., 2007) and levels (1.88 to 4.59 %) in cereals from Tanzania (Towo *et al*., 2006). The difference may be due to high levels of protein in sesame seeds (20/100 g) and ascorbic acid in pumpkin seeds (1.9 mg/100g). Ascorbic acid and protein influence bioavailability of elements in a dose dependent way, by forming soluble complexes with the elements, thus facilitating their absorption (O' Dell, 1984; Hallberg and Hulthĕn, 2000; Kies and McEndrea, 1982).

Bioavailability of zinc, chromium, selenium and iron estimated by algorithms and those obtained by *in vitro* digestion procedure were compared and results presented in Table 4.25.

Table 4.25: The percentages of bioavailable zinc and iron from *in vitro* digestion procedure, algorithms and WHO/FAO (2004) estimates

Parameter	Zinc	Iron
In vitro digestion procedure	34.4	24.2
Algorithm estimate	13.9	1.22
WHO/FAO estimate	10.0	5.0

The algorithm model predicted lower zinc (13.9 %) and iron (1.2 %) absorption as compared to the *in vitro* studies (34.4 % and 24.2 %, respectively) and WHO/FAO (2004) estimate for

iron (5 %) and zinc (15 %) in low bioavailability diet, where phytate to zinc molar ratio is above 15. Phytate inhibits both zinc and iron in a dose dependent manner and risks of zinc and iron deficiency increase significantly at molar ratio of 15:1 and 0.15:1 of phytate to zinc and phytate to iron, respectively (Fredlund *et al.*, 2006; Hurrell *et al.,* 1992; Ferguson *et al.*, 1989). This is because phytate has 12 replaceable portions and high density of negatively charged groups (6 phosphates), which allows it to form insoluble chelates with multivalent cations, such as Ca^{2+}, Zn^{2+}, Cr^{3+}, and Fe^{3+} (Herland and Oberleas, 1987).

The difference in the algorithm estimates and WHO/FAO (2004) values is due to use of different parameters in the two models: Miller *et al.* (2007) method models zinc absorption as a function of the number of millimoles of phytate and zinc in the food; whereas WHO/FAO (2004) method, models zinc absorption as a function of a range of phytate/zinc molar ratio in the food. The later prediction is not specific to a given food. A part from the inhibitory effects of phytate, both Miller *et al.* (2007) and WHO/FAO (2004) models do not factor effects of other food components, such as calcium and amino acids which are also known to influence bioavailability of zinc (Lönnerdal, 2000).

The higher dialyzable zinc (34.4 %) and iron (26.4 %) in the *in vitro* digestion procedure could be due to enhancing of effect of protein (20 mg/100g) in sesame seeds that was not factored in the two algorithms. However, *in vitro* digestion procedure used in this study estimated only dialyzable ions and the result may be slightly above the actual absorption, because, not all dialyzable ions are absorbable. This error can be reduced by incorporating caco-2 cells to estimate uptake of the dialyzable ions. On the other hand, *in vitro* digestion procedure may underestimate absorption of nutrients in a nutrient-depleted person, because, it does not factor the role of physiological homeostasis that regulates absorption of the trace

elements. This is because, in a nutrient-depleted person, the amount of nutrient absorbed is influenced by secretion of absorption enhancers within the gastrointestinal system or increase in the number of receptor sites in the intestinal mucosal cells (Campen and Glahn, 1999).

The algorithm for iron absorption developed by Hallberg and Hulthĕn (2000) allows for adjustment of nutrient status of the subjects, while the current and widely used zinc absorption algorithms do not factor the effect of nutrient status of the subject on absorption (Miller *et al*., 2007; WHO/FAO, 2004; Brown *et al*., 2004). Although subject's nutrient status is factored in the algorithm used for estimating iron absorption by using the borderline iron deficient status, the results may not be accurate because it assumes that iron absorption changes linearly with change in nutrient status (Hallberg and Hulthĕn, 2000; Campen and Glahn, 1999).

Both algorithm model and *in vitro* digestion procedure do not incorporate other host related factors, such as pathological state, age, gender and body weight; which influence absorption of the elements. Therefore, the results obtained using these methods may not be generalizable to all subjects (Gibson, 2007). The correct bioavailability of the elements can be obtained only when *in vivo* experiment is carried out in human beings. Nevertheless, *in vitro* digestion procedure is still a good predictor of relative nutrients bioavailability and thus useful in investigating role of food matrix on bioavailability of nutrients (Miller and Schricker, 1982).

4.6.4 Bioavailability of the nutrients in food supplement

Finger millet, sesame and pumpkin seeds were used to prepare food supplement because, they contained high levels of selenium, zinc, iron and chromium. Table 4.26 shows nutrients

composition of the food supplement made by mixing sesame seeds, pumpkin seeds and finger millet.

Table 4.26: The levels of nutrients in millet grains, pumpkin and sesame seeds in the food formulation

Nutrients	Sesame seeds	Millet grains	Pumpkin seeds	Concentration in food formulation (200g, ratio of 4:4:1)
Iron (mg)	15.20	8.10	3.10	26.40
Zinc (mg)	3.70	1.50	2.00	7.20
Chromium (mg)	0.022	0. 58	0.056	0.66
Selenium (mg)	0.025	0.038	0.014	0.077
Energy (K cal)[a]	508.80	291.30	96.60	896.70
Protein (mg) [a]	17.76	13.60	4.30[b]	35.70
Phytate (mg)[a]	506.88	190.96	265.23	963.07
Calcium (mg)	865.80	244.20	21.50	1131.50
Ascorbic acid (mg)[a]	0.00	0.00	0.42	0.42

[a] adapted from Sehmi (1993)

The food formulation consisted of millet, sesame and pumpkin seeds, combined in respective ratio of 4:4:1. The ratio was arrived at in order to prepare a formulation containing high levels of the elements. Millet provided the highest level of chromium (0.58 mg/88.9 g) and selenium (0.038 mg/88.9 g), whereas sesame seeds provided the highest level of zinc (3.7 mg/88.9 g), energy (508.8 kcal/88.9 g), protein (17.76 mg/88.9 g) and iron (15.2 mg/88.9 g). Level of selenium (0.077 mg/200 g) in the supplement was above RDA for adult male (0.034 mg) and female (0.026 mg) (FAO/WHO, 1998). Chromium level (0.66 mg) in the

supplement was also above the USA adequate intake for adult male (0.035 mg) and female (0.025 mg) (FNB, 2001).

In low zinc bioavailability diet (15 %), WHO/FAO (2004) estimates an individual normative requirement for zinc to be 119 and 144 μg/kg body weight per day for adult female and male, respectively. When this is applied to the study subjects with a mean weight of 59.6 kg (male) and 55.9 kg (female), their average normative zinc requirements are 8.6 mg (male) and 6.7 mg (female). Therefore, the supplement could meet 83.7 % and 107 % of the daily zinc requirements for the adult male and female subjects, respectively.

However, the supplement could meet a higher percentage of zinc RDA than estimates based on WHO/FAO (2004). This is because, higher levels of dialyzable and bioavailable zinc estimates were obtained from *in vitro* digestion (34.4 %) and algorithm (30.8 %), respectively. Although higher zinc:phytate molar ratio (12.5) and (calcium×phytate):(zinc) molar ratio (355.3) may result in negative interaction that could depress zinc absorption (Hurrel *et al*., 1992; Ellis *et al*., 1987); such effects could be reduced by high level of protein (35.7 mg) in the supplement. Protein contains peptides and amino acids that form soluble complexes with zinc, therefore enhancing its absorption (Velasco-Reynold *et al*., 2007). Furthermore, a large proportion of the subjects (95.8 %) had higher serum zinc levels (> 70 g/dL) indicating adequate zinc intake, despite low levels of zinc (0.6 to 3.5 mg/100g) observed in the foods that make up a large proportion of the subjects' diet (68 %), such as cereals, vegetables and tubers. This indicates that zinc contained in the foods may be of higher bioavailability as shown by the *in vitro* studies (34.4 %) or its bioavailability in the foods is enhanced by the food preparation methods of the subjects.

When zinc bioavailability estimate (34.4 %) from *in vitro* digestion procedure is applied to the subjects, based on normative zinc requirements of 1.4 mg/day (male) and 1.0 mg/day (female) (WHO/FAO, 2004), the supplement that contained 7.2 mg/200g of zinc could meet 177 % and 248 % of the daily zinc requirements for male and female subjects, respectively. Therefore, 115 and 81 g of the supplement could meet daily zinc needs of the male and female subjects, respectively.

Intake of zinc (10 mg/day) is associated with reduced (50 %) watery diarrhoea among HIV-positive children as compared to placebo, whereas zinc intake of 45 mg/day decrease production of pro-inflammatory cytokines and oxidative stress that increase rate of HIV replication (Bobat *et al*., 2005; Prasad *et al*., 2004; Beck *et al*., 2003). The immune malfunction of PLHWA is partly caused by imbalance between Th1 and Th2 cells that is caused by zinc deficiency (Mosmann and Coffman, 1989; Prasad, 2007). This is because zinc deficiency reduces CD4:CD8+ ratio, helper T-cells functions and promotes Th-2 response at the expense of Th-1 (Prasad, 2007). The Th-2 provides humoral immunity that is ineffective in fighting viral infections and enhances the ability of virus such as HIV to infect other cells (Riera *et al*., 2003).

On average, basal and menstrual iron lose in adult woman is 14 µg/kg/day and 0.51 mg/day, respectively (Green *et al*., 1968). When this is applied to the female subjects who had a mean weight of 55.9 kg (female), the average daily iron requirement would be 1.2926 mg. Based on low iron bioavailability diet (5%) for the developing countries, this translates into 26 mg of iron RDA for the female subjects.

The only nutritional iron requirement in male adults is for replacement of basal iron lose, which is estimated to be 14 µg/kg/day (Green *et al*., 1968). Therefore, the daily iron requirements for the male subjects with a mean weight of 59.6 kg, would be 0.83344 mg/day. In low iron bioavailability diet (5 %) as suggested by WHO/FAO (2004) for the developing countries, the average daily iron requirements for the male subjects would be 16.7 mg. The supplement with iron level at 26.4 mg/200 g could therefore, meet 101 % (female) and 158 % (male) daily iron requirements, based on low iron bioavailability diet (5 %) (WHO/FAO, 2004).

Furthermore, the level of dialyzable iron from the *in vitro* study (24.6 %) indicates that, the supplement could meet a higher percentage of iron RDA than the estimates (101% and 158 %) from WHO/FAO (2004). The 26.4 % of dialyzable iron is equivalent to 6.97 mg of iron in the supplement that is available for absorption by simple diffusion and carrier mediated process. When this is applied to the female subjects with iron daily requirement of 1.2926 mg as calculated before, the supplement could meet 5 x RDA of the female subjects. For the male subjects with iron requirements of 0.83344 mg/day as calculated before, the supplement could meet 8 x RDA of the male subjects. When the range of individual variation (±15 %) in iron absorption as estimated by FAO/WHO (1998) is factored, the supplement could meet 4.7 x RDA and 7 x RDA of iron daily requirement for the female and male subjects. Therefore, 30 and 43 g of the supplement could meet daily iron requirements of the male and female, subjects respectively.

Although adequate levels of iron (6.2 to 10.7 mg/100g) were observed in the foods that form a large proportion of the subjects' diet (68 %), such as cereals, vegetables and tubers, a large proportion of the subjects (male: 35 % and female: 45) were still suffering from anaemia (<

11 g/dL) and anaemia related clinical conditions such as pallor (15 %) and fatigue (50 %). This indicates that either iron contained in the subjects' diet is of low bioavailability or the food preparation methods or eating habits of the subjects decrease bioavailability of iron in the foods (Walingo, 2009). However, the food formulation prepared contained higher bioavailable iron as shown by the *in vitro* studies (24.2 %), therefore, when used may reduce prevalence of iron deficiency anaemia and its related clinical conditions observed among the subjects.

The level of selenium in the supplement (77 µg/200 g) is above WHO/FAO (2004) selenium RDA for male (34 µg/day) and female (26 µg/day). From the selenium absorption model derived in this study ($y = 50.963 + 0.148x$), 62.4 % of the total selenium in the supplement will be absorbed by the subjects.

Intake of high levels of selenium (0.2 mg/day) is known to reduce the rate of decline in CD4+ cells and risks of hospitalisation (Burbano *et al*., 2002). Although no study has associated intake of selenium supplement with decreased viral load, a study reported suppressed progression of HIV-1 viral burden and increased CD4+ cell counts with intake of 0.2 mg/day of selenium (Hurwitz *et al*., 2007). This is because; high intake of selenium reduces reactive oxygen species that possibly alter a viral genome, such that a mildly pathogenic virus becomes highly virulent (Beck *et al*., 2003).

The HIV-1 encodes homologues of selenoproteins that influence immune–related genes and regulate cytokines production and cellular proliferation; possibly the activity of the selenoprotein could be suppressing HIV-1 replication in mechanism that is still not clear (Mckenzie *et al*., 2002; Taylor *et al*., 2000). High selenium intake also reduces the

abnormally high levels of IL-8 and TNF-α found in PLWHA, which are associated with neurological damage, Karposis Sarcoma, wasting syndrome and increased viral load (Baum *et al*., 2000). Although HIV could use high level of selenium in the host for its replication, high selenium status in the host is necessary for optimum activity of glutathione peroxidase enzyme that removes reactive oxygen species, which damage tissues and induces HIV-1 gene activation and replication (Woods, 1999; Patrick, 1999).

There is need for balanced intake of iron by PLWHA because, both iron deficiency and overload increase rate of progression of HIV and AIDS. Elevated iron status induces production of reactive oxygen species, which alter and suppress immune response. High levels of reactive oxygen species are associated with increased mRNA levels of pro-inflammatory Th2 response cytokines and chemokines and decreased anti-inflammatory cytokines IF-y and IL-2. High levels of the pro-inflammatory and low levels of anti-inflammatory cytokines enhance viral replication, resulting in selection of a more pathogenic HIV strain (Beck *et al*., 2003). On the other hand low iron status that is indicative of anaemia is associated with fatigue, rapid progression to AIDS and increased risks of death (O'Bren *et al*., 2005; Semba and Gray, 2001).

The major cause of anaemia among PLWHA is still unclear, as to whether it is an inflammatory response or an increased iron demand caused by high rate of immunity cellular turnover resulting from HIV infection (Doherty, 2007). A study associated use of Highly Active Antiretroviral Therapy (HAART) with increased HGB levels, which was attributed to reduced levels of pro-inflammatory cytokines and improved immunological status of PLWHA (Sarcletti *et al*., 2003). This is because pro-inflammatory cytokines such as TNF-α and IF-γ induce withdrawal of excess iron needed by microbial pathogens for growth, thus

inhibiting red blood cells formation because; iron is channelled to liver, resulting in anaemia (Doherty, 2007). In another study, use of HAART had no effect on HGB levels (Rousseau *et al*., 2000), while intake of iron supplement (18 mg/day) reduced anaemia and improved iron status, but did not have significant effect on HIV load (Semba *et al*., 2007).

Despite the uncertainty on cause of anaemia, it is apparent that PLWHA are at risk of anaemia and may benefit from adequate intake of iron. For example, a modest intake of iron (18 mg/day) increased HGB levels within the low end of normal range, improved quality of life and physical functioning of PLWHA (Semba *et al*., 2007). Therefore, level of iron in the food supplement (26.4 mg/200 g) is safe and may increase low HGB levels in subjects (male = 11.3 and female = 11.2 HGB), reduce high level of fatigue (50 %) and strengthen immune system of the subjects.

Although benefits of chromium supplementation are yet to be confirmed on large randomized clinical trial (FNB, 2001), intake of chromium supplement (above 0.2 mg/day) reduced body fat, increased lean body mass, lowered insulin and improved lipid metabolism (EVM, 2002). High levels of chromium (0.66 mg) in the supplement could therefore, reduce underweight amongst the subjects (men = 23.5 % and women = 10.8 %) and other problems facing PLWHA, such as lipid abnormalities and insulin resistance (Tang *et al*., 2005).

In general, intake of the food supplement could improve nutritional status of the trace elements in PLWHA therefore, making them available for use as co-factors of antioxidants. The antioxidants strengthen immune system of PLWHA, by removing reactive oxygen radicals that enhance viral replication (Staal *et al*., 1993) and damage cells participating in innate immunity (Erickson *et al*., 2000). Use of the supplement therefore, could strengthen

immunological status of the subjects, and consequently delay progression of their infection and early use of ARVs.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The questionnaire results indicated very few subjects smoked (2.8 %), took alcohol (5.7 %) and used food supplements (3.8 %). More than a half of the subjects (65.1 %) could not afford foods that they would like to eat and 73.3 % were taking less than three meals a day. Starch, proteins, animals and plants food sources accounted for 45.17 %, 21.9 %, 17.48 % and 73 %, respectively of total foods consumed by PLWHA in Butula Division.

The study indicates that some indigenous foods which are widely used in Butula Division, contain sufficient levels of chromium (0.23 mg/100g) and selenium (0.036g/100g) that can meet RDA for adult person, but moderate levels of iron (10.7 mg/100g) and low levels of zinc (2.3 g/100g) that can meet 39.6 and 24. 5 % male and 18.2 and 35.9 % female adult RDA for iron and zinc, respectively, based on low iron (5 %) and zinc (15 %) bioavailability diet recommended for the developing countries (WHO/FAO, 2004).

More than a half of PLWHA (77.3 %) in the study had normal weight (BMI = 18.5 to 24.9), while 61.0 % had anaemia (HGB<11 g/L). The highest proportion of the subjects (53.6 % male and 71.3 % female) had CD4+ cell count above 500 cells/mm^3, whereas the lowest proportion (6.3 % male and 6.9 % female) had CD4 + cell count below 200 cells/mm^3. Low levels of CD4+ cells was associated with low levels of MCH (r = 0.262, p> 0.01) and MCHC (r = 0.243, p> 0.05). Herpes zooster and Kaposis Sarcoma infections were associated with high levels of RBC (r = 0.212, p = 0.017 and r = 0.242, p = 0.01, respectively) and HCT (r = 0.184, p = 0.039 and r = 0.177, p = 0.046, respectively).

Very few subjects (4.2 %) had low serum zinc levels indicative of zinc deficiency (< 70 g/dL) and the mean serum zinc level for the male subjects (354.7 μg/L) was significantly ($p = 0.006$) higher than the levels for the female subjects (279.7 μg/L). Low level of CD4+ cells was associated with high level of serum zinc ($r = -0.195$, $p = 0.055$), whereas several clinical conditions such as headache ($r = -0.18$, $p=0.047$), fever ($r = -0.195$, $p = 0.035$) and loss of appetite ($r = -0.245$, $p = 0.007$) were significantly associated with low serum zinc levels.

In vitro digestion procedure indicated higher levels of bioavailable zinc (34.4 %) and iron (24.2 %) than predicted from the algorithms for zinc (13.8 %) and iron (1.2 %). *In vitro* digestion procedure gave a better prediction of bioavailable elements than algorithms because it factors all nutrients in the food, whereas algorithm factors only a few nutrients in the food.

5.2 Recommendations

1. Bioavailability of the trace elements in the usual diets of PLWHA could be improved by increasing proportion of animals food sources from the current 21.9 % to 50 % to raise levels of proteins, and decreasing proportion of plants food sources from 73 % to 50 % to reduce levels of inhibitors such as phytates and polyphenols.
2. Most PLWHA rely on their farms as source of food (56.5 %) and therefore need incentive policies and better farming systems, which promote the development, availability, distribution and use of indigenous foods especially the vegetable seeds, which contain high levels of selenium (0.05 mg/100g), chromium (0.22 mg/100g) and zinc (3.5 mg/100g).
3. Low levels of CD4+ cell counts were associated with low MCH levels ($r = 0.262$, $p> 0.01$), while herpes zooster and Kaposis Sarcoma infections were associated with high RBC levels ($r = 0.212$, $p = 0.017$ and $r = 0.242$, $p = 0.01$, respectively). Therefore, it

is important for medical personnel to advice PLWHA with low levels of CD4+ cell counts the need to take foods with high levels of iron, while those with herpes zooster and Kaposis Sarcoma infections need to take foods with low levels of iron.

4. Prevalence rate of anaemia among male PLWHA was moderate (35.29 %), while among the female PLWHA (45.05 %) was severe and therefore, PLWHA should be adviced to take food containing high levels of iron.

5.3 Areas for further study

1. Different species of the indigenous foods from different parts of the country need to be analysed to determine geographical and interspecies variation of levels of the trace elements in the foods.
2. There is need for improving algorithm for estimating bioavailable zinc, since the current ones: WHO/FAO (2004) and Miller *et al.* (2007) do not factor the effects of zinc status, calcium and iron, which have been shown to influence absorption of zinc. Algorithms for estimating bioavailable selenium and chromium need to be developed, as such algorithms are useful in a poor resource set-up where *in vitro* and *in vivo* studies cannot be carried out.
3. Effects of high levels of chromium and co-factor of antioxidant elements (selenium, zinc and iron) in the food supplement on insulin levels in diabetics and high oxidative stress subjects (PLWHA) need to be examined with *in vivo studies.*
4. An intervention study using food supplement prepared in the study should be carried out to PLWHA to determine the actual uptake of the trace elements.

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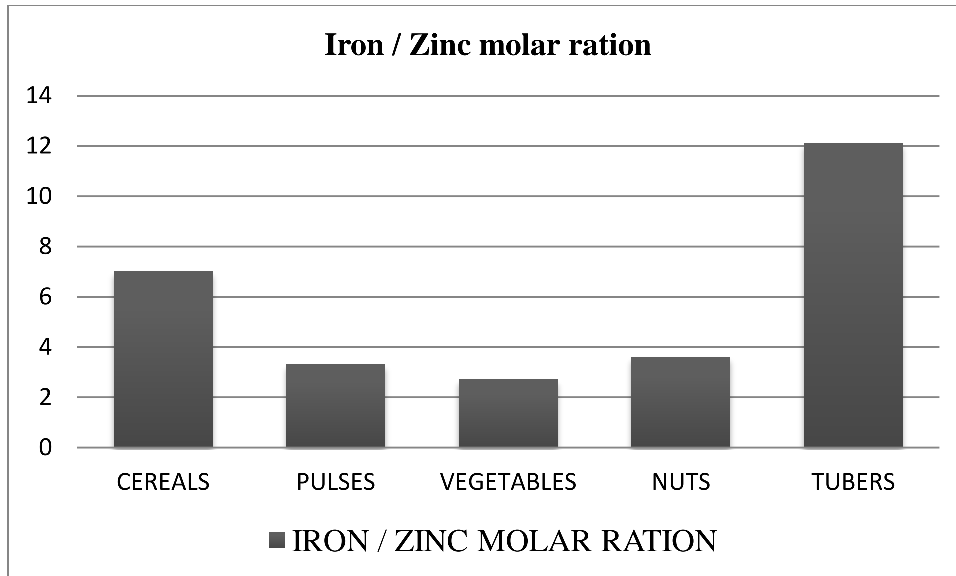
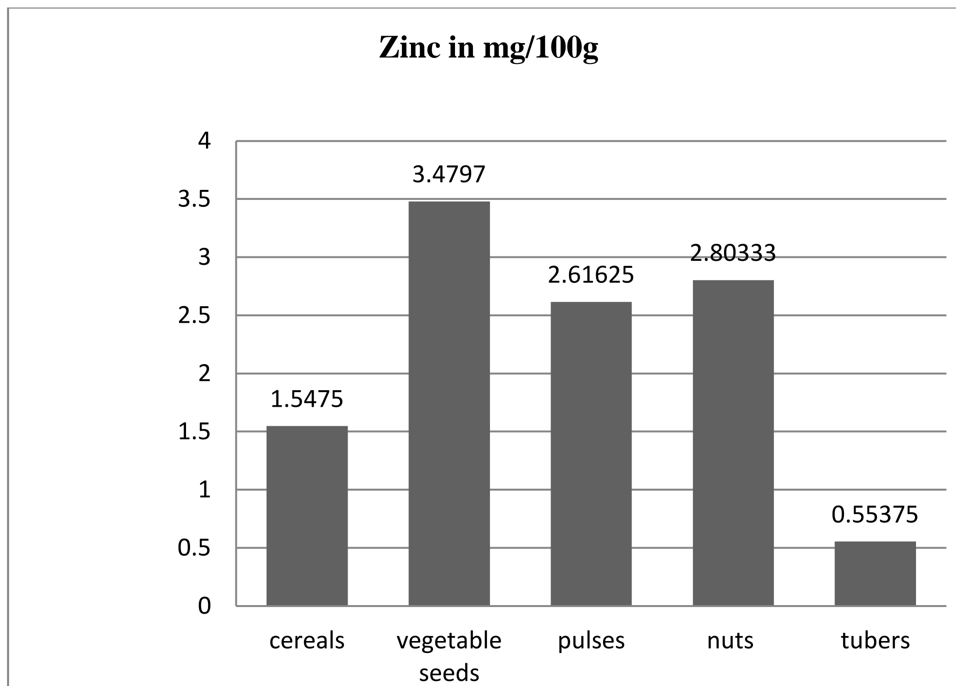
APPENDICES

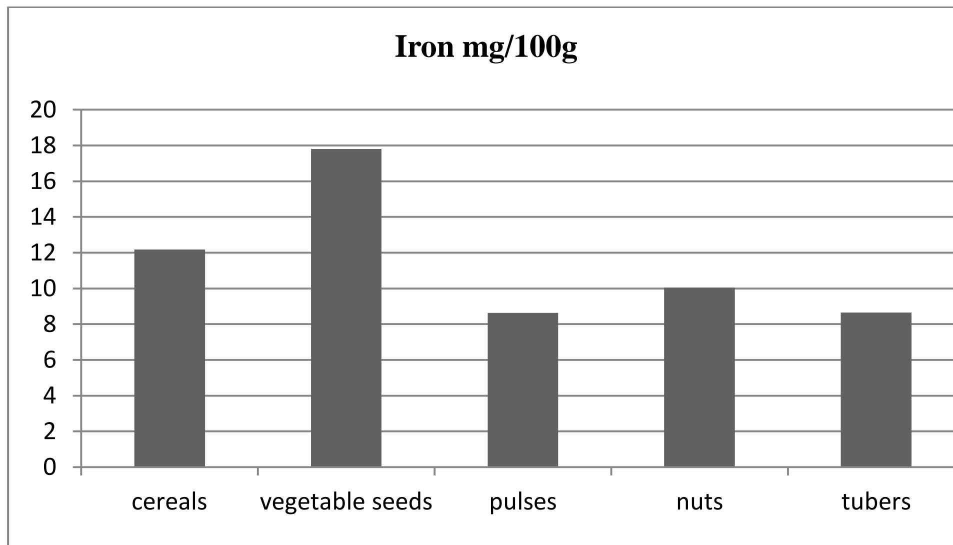
Appendix 1 Levels of iron / zinc in the the grains analysed



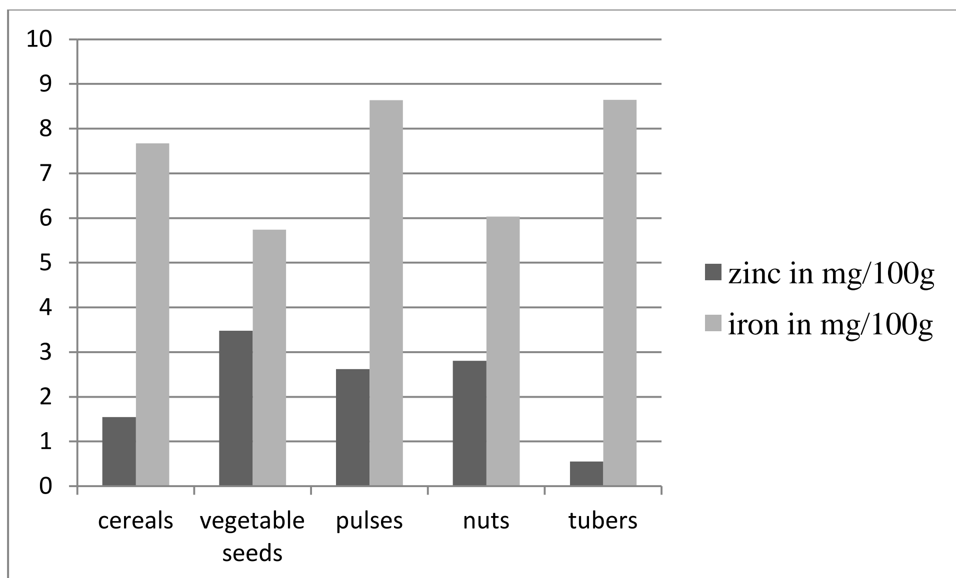
Appendix 2 Levels of zinc in the foods analysed



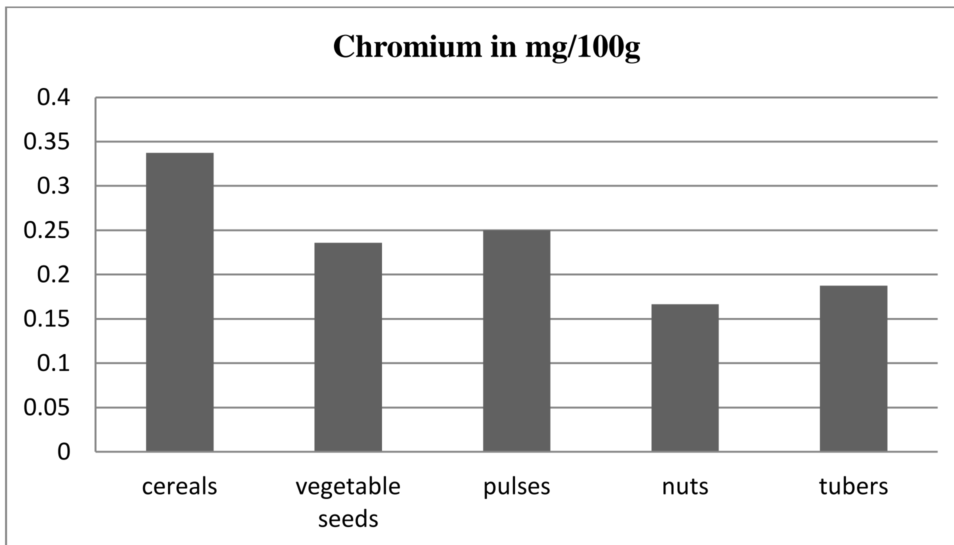
Appendix 3 Levels of iron in the foods analysed



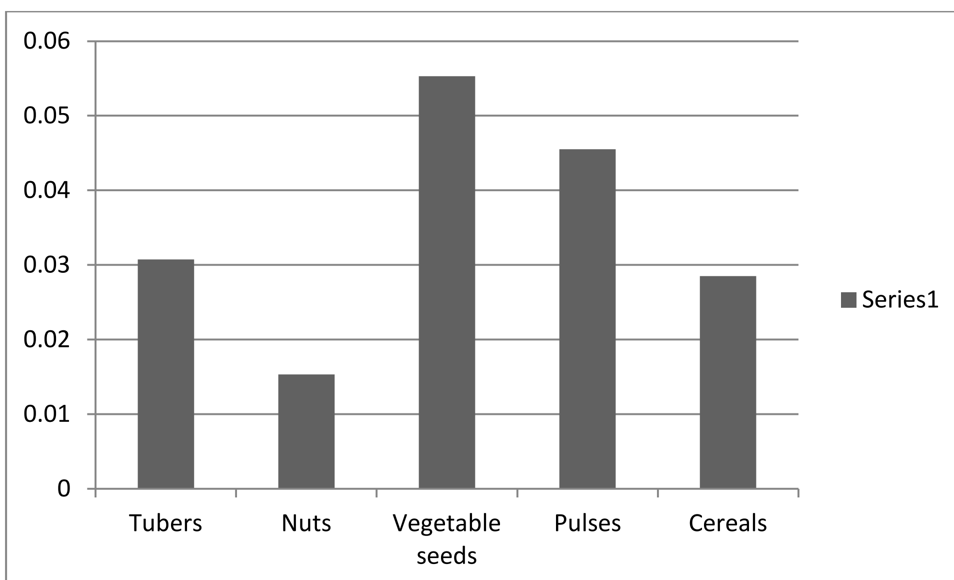
Appendix 4 Comparison on levels of the trace elements in the foods analysed



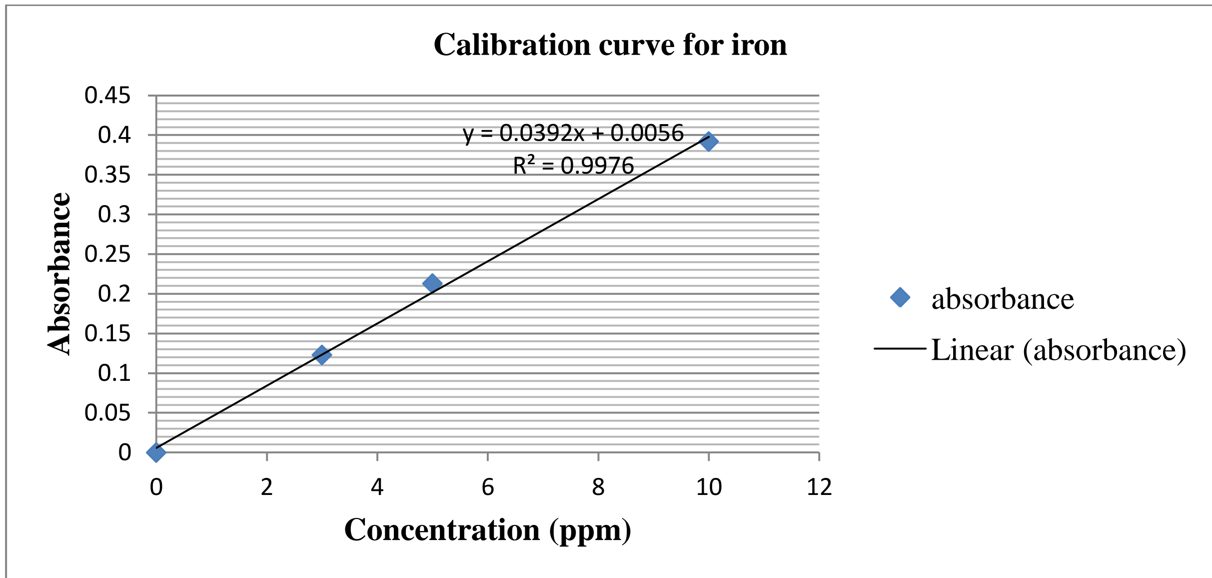
Appendix 5 Chromium levels in the foods analysed



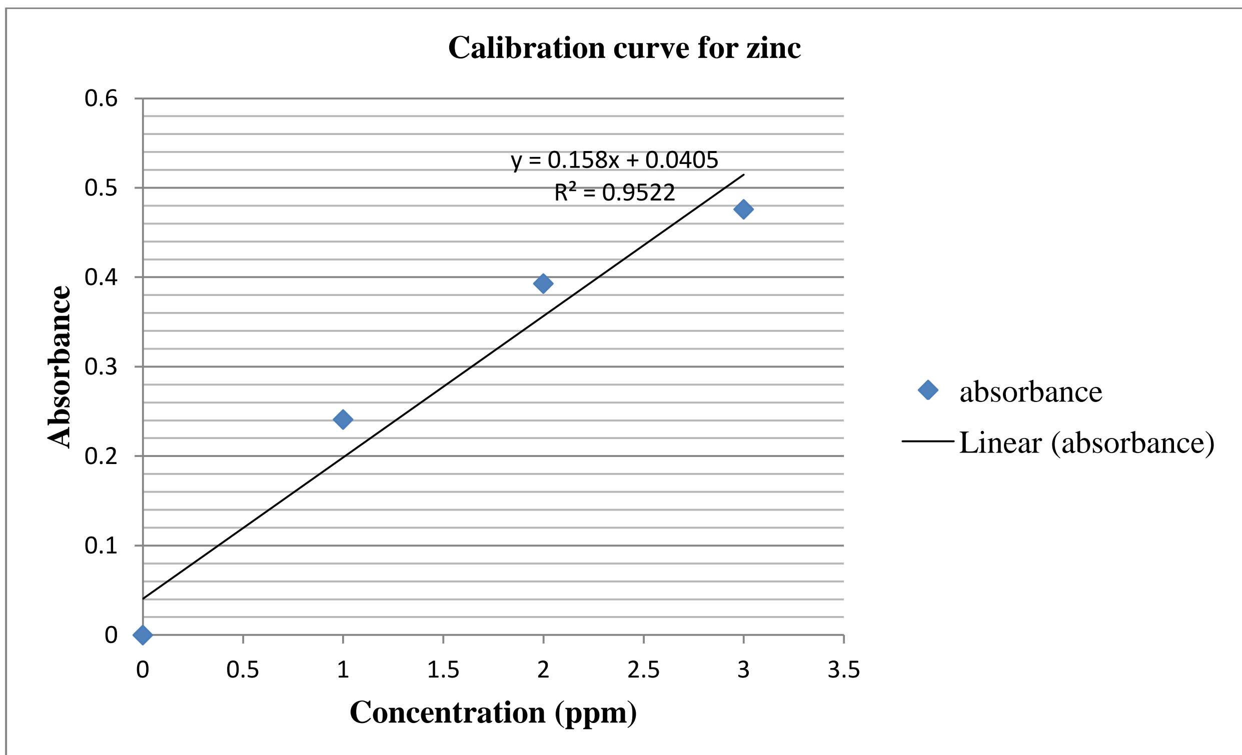
Appendix 6 levels of selenium in the foods



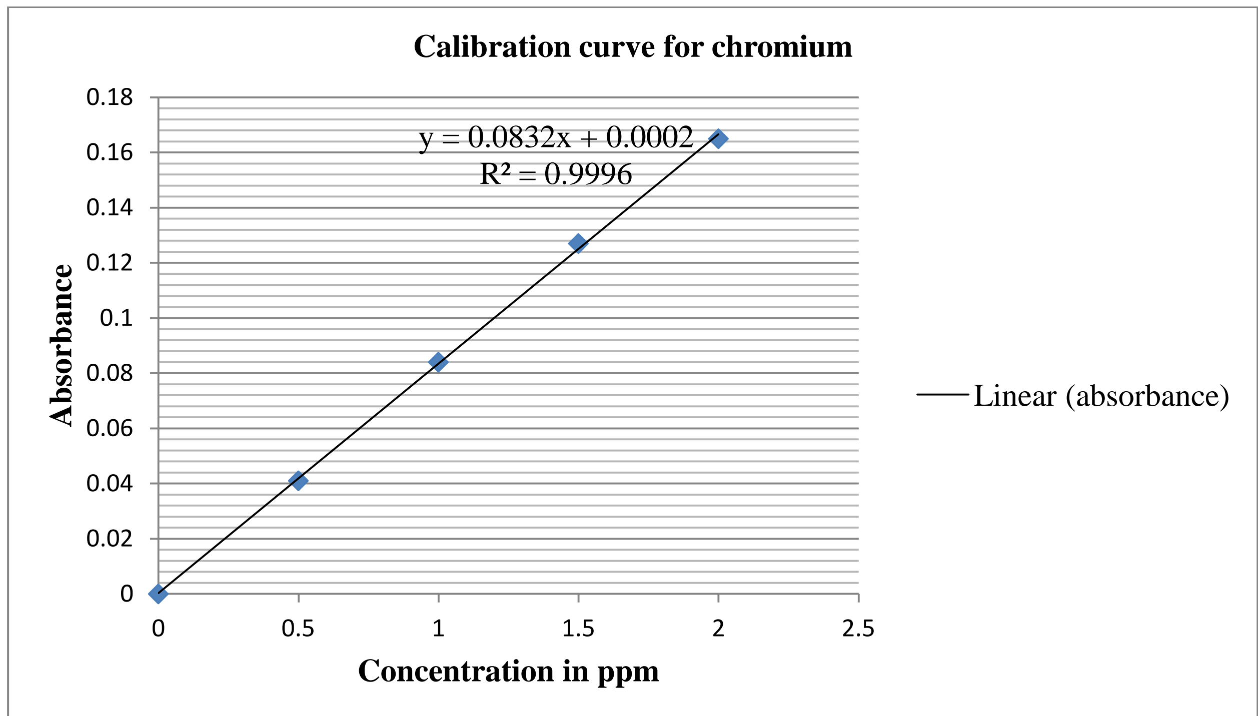
Appendix 7 Calibration curve for iron



Appendix 8 Calibration curve for zinc



Appendix 9 Calibration curve for chromium



Appendix 10 Main sources of dishes consumed by PLWHA in the study

Type of foods	Foods	Percentage of food accompaniment of main dish in household 1	Percentage of food accompaniment of main dish in household 2	Average of percentages of food accompaniments of main dish in household 1and 2
Cereals	Ugali	0.0	0.4	0.2
	Porridge	0.0	1.9	0.95
	Maize	1.5	0.2	0.85
	Rice	0.4	0.4	0.4
	Chapatti	0.0	0.0	0.0
	Total	01.9	2.9	2.4
Tubers	Potatoes	1.5	0.8	1.15
	Sweet potatoes	0.4	0.4	0.4
	Yams	0.0	0.0	0.2
	Cassava	0.0	0.4	0.0
	Nduma	0.0	0.0	0.0
	Total	**1.9**	**1.6**	**1.75**
Fruits	Bananas	0.0	0.8	0.4
	Avocado	0.4	0.0	0.2
	Total	0.4	0.8	0.6
Plant proteins	Beans	1.2	1.9	1.55
	Peas	0.0	0.4	0.2
	Total	**1.2**	**2.3**	**1.75**

Continuation of appendix 10

Type of foods	Foods	Percentage of food accompaniment of main dish in household 1	Percentage of food accompaniment of main dish in household 2	Average of percentages of food accompaniments of main dish in household 1and 2
Nuts	Groundnut	0.4	0.0	0.2
	Total	**0.4**	**0.0**	**0.2**
Animal proteins	Meat	0.8	0.4	0.6
	Fish	6.4	32.3	19.35
	Pork	0.4	0.0	0.2
	Milk	0.4	0.0	0.2
	Eggs	0.0	0.8	0.4
	Total	**8**	**33.5**	**20.75**
Vegetables	Cabbages	0.8	1.6	1.2
	Sukuma wiki	2.7	2.7	2.7
	Kunde	35.0	16.4	25.7
	Managu	0.8	1.5	1.15
	Miro	1.5	6.8	4.15
	Mrende	0.8	3.8	2.3
	Saka	0.8	1.5	1.15
	Veges	40.0	10.7	25.35
	Pumpkin	0.0	1.5	0.75
	Tomatoes	0.0	0.4	0.2
	Total	**82.4**	**46.9**	**20.75**

Foods	Source of main dish %	Source of accompaniment of main dish %	Snacks %	Average %
Cereals	53.05	2.1	47.6	34.25
Tubers	29.3	1.75	1.7	10.92
Pulses	2.1	2.15	9.0	4.42
Animal food sources	0.0	20.75	31.7	17.48
Vegetables	0.0	63.2	0.4	21.2
Nuts	0.0	0.2	1.2	0.047
Beverage	0.0	2.1	2.3	2.2
Others	14.1	6.1	2.3	7.5
Total	99.5	98.95	99.3	99.59

Appendix 11 Algorithms for Bioavailability of iron

Effect of phytate

Equation 1

$$\boldsymbol{Log\ aborption\ ratio\ (with\ or\ without\ phyate) = -0.30 \times log(phytate\ P + 1)(in\ mg)}$$

Effect of calcium

Equation 2

$$Iron\ absorption\ (mg) = 0.4081 + [\frac{0.5919}{1 + 10^{-[2.022 - \log(Ca+1)] \times 2.919)}}]$$

Effect of ascorbic acid

Equation 3

$$absorption\ ratio = 1 + 0.01AA\ (in\ mg) + \log[phytate - p\ (in\ mg) \times 0.01 \times 10^{0.8875 \times \log(AA+1)}$$

Equation 4

Total bioavailable iron = factor of phyate × factor of ascorbic acid × factor of calcium × basal factor of 22.1

Adopted from Hallberg and Hulthèn, 2000

Appendix 12 Algorithm for determination of available zinc

$$\mathbf{TAZ = 0.5}\left\{ A_{max} + TDZ + K_R \,.\left(1 + \frac{TDP}{K_P}\right) - \sqrt{\left(A_{max} + TDZ + K_R \,.\left(1 + \frac{TDP}{K_p}\right)\right)^2 - 4.\, A_{max} + TDZ} \right\}$$

(Miller *et al*., 2007) **Equation 4.2**

Appendix 13 Foods liked by PLWHA in the study

Foods	Percentage %
Bananas	0.4
Beans	4.6
Cassava	2.3
Chapati	1.9
Eggs	0.4
Fish	1.1
Githeri	3.4
Milk	0.4
Mrende	0.4
Nduma	0.4
Potatoes	3.1
Sweet potatoes	1.5
Rice	5.3
Tea	0.4
Ugali	69.6
Ugali fish	0.4
Uji	0.4

Appendix 14 Information and consent form

(To be translated into Kiluhya)

I am a student at Kenyatta University in the department of Chemistry. I am carrying out a study on bioavailability of immunity boosting trace elements, for a partial fulfilment of the requirements for the award of Master of Science degree. People living with HIV and AIDS commonly suffer from malnutrition due to chronic diarrhoea, anorexia, mal-absorption, impaired nutrient storage, increased energy demands and altered metabolism. Malnutrition increases oxidative stress and suppresses immune system, conditions which lead to increased HIV replication and diseases progression, drug inefficiency and resistance. If supplementation is started at an early stage, HIV disease progression may be reduced consequently delaying use of ARVs. Some indigenous foods contain the nutrients at high levels, that can slow progression of the disease and we want to determine their effectiveness.

I would like you to participate in the study. You will be required to provide some information on your nutrition, health, family and source of income. You will be given food supplements to eat, and levels of trace elements, viral load, and CD3+T, CD4+T, CD8+T and CD16/CD56+ NK cell counts in your blood (10ml) will be determined every month for 3 months. During the study period you will be required to go to the local hospital for medical check up. The check up will enable me determine the effects of food formulation on your health. You will be informed of any important health issue relating to special medical needs by referring you to the appropriate referral hospital. You are free to withdraw from the study at any stage and your withdrawal would not affect your rights in anyway. However, your full participation and following study rules will contribute highly to the success of this study. The

information obtained from you will be treated strictly as confidential and will not be used for trade or politics. No rewards will be given to you for participating in the study. Please feel free to ask any question on any issue that is not clear to you, after you have read and had contents of the consent form explained to you. Please put your fingerprint or sign the following statement if you are willing to participate in the study.

I, the undersigned have understood the above information, which has been explained to me by the investigator. I had opportunity to ask questions, all of which were answered to my satisfaction. I understand that my participation is on voluntary: I can withdraw from the study if I wish at any stage without further explanations. Therefore, I freely confirm my availability to be involved in the study.

Name …………………………… …………………………….

Finger print or signature ………….. Date

Appendix 15 Recruitment questionnaire

Name of Interviewer ....

Date of interview..

Questionnaire No. ....

SECTION A. Personal history
Code No.. ……………………………………………………………………….

Physical Address/Telephone No. …………………………………………………

(**Tick appropriate answer)**

1. Sex: female (1) male (2)

2. Date of birth …………………………………………………………….._

3. Marital status……………………………………………………………..

4. State your occupation…………………………………………………………..

5. State occupation of your spouse. ………………………………………………

6. How much do your household spend on food per month?…………………….

7. Do you use cosmetics? Yes (1) No (2)

If yes specify ……………………………………………………………….

8. State area of residence ……………………………..…………………..

9. Status of residential area: Urban (1) suburban (2) industrial (3) farm (4)

lakeshore (5)

10. How long have you leaved in the current place of residence…………………..

11. How many people live in your household…………………………………

12. List people living in your household in the table below using codes provided.

Name (optional) A	Age B	Relation to subject C	Level of education D	Occupation E
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

B >5yrs (1) 5-10yrs (2) 11-18 (3) <18 (4)

C Spouse (1) Child (2) Parent (4) Grandparents (5) House help (6) Others (7) specify……………………….

D Pre-Primary (1) >STD 4 (2) >STD 4-8 (3) secondary level (4) college level (5)

E Self employed (1) Formal employed (2) Not employed (3) Others specify (4)

SECTION B: Dietary habits

13. Are you on a special/ recommended diet? Yes (1) No (2)

If yes, specify…………………………………………………………….

14. Do you use supplement with vitamins or minerals? Yes (1) No (2)

If yes specify ……………………………………………………………

15. Describe your appetite: Good (1) Fair (2) Poor (3)

16. Where do you get your foods? Market (1) Own farm (2) Donation (3)

17. Which non-alcoholic drink do you use? Cocoa (1) Coffee (2) Tea (3) Soya (4) other (5),

specify………………………………………

18. State amount used everyday (e.g. No. Of cups)……………………………..

19. Are you allergic to some foods? Yes (1) No (2)

If yes, state them ………………………………………………………….

20. Do you drink alcohol? Yes (1) No (2) Formerly (3)

If yes, specify brand and amount……………………………………………….…….

If formerly, state i) when you stopped………………………………………………

Ii) Brand………………………………………………………….

21. Do you smoke? Yes (1) No (2) Formerly (3)

If yes, state i) type and brand……………………………………………………

ii) number used per day…………………………………………………

If formerly, state i) when you stopped……………………………………………

ii) Brand……………………………………………………..…

22. List foods used frequently

Foods	Frequency
1	
2	
3	
4	
5	
6	
7	
8	

Everyday (1) more than once a week (2) once a week (3) monthly (4)

Occasionally (5)

SECTION C: Medical details

(To be filled by physician)

23. Diagnosed illness………………………………………………………………

24. Chronic illness…………………………………………………………………

25. Specify drugs used ………………………………………………………………

24. How long have you lived with your HIV status?

>1year (1) 2-3 years (2) 4-5 years (3) 6-10 years (4) >10 years (5)

25. HIV/AIDS stage according to CDC criteria (1) (2) (3) (4)

26. How do you manage your HIV/AIDS status?

Use of vitamins and mineral supplements (1)

Use of ARVs (2)

Use of prophylaxis (3)

Others (4), specify…………………………………………………….

27. Do you use contraceptive or other hormonal treatment? Yes (1) No (2)

If yes specify oral contraceptive/or other hormonal treatment in past one year……………………………………………………………………………………

28. Have you undergone surgical operation, implant, prostheses and amalgams? Yes (1) No (2). If yes, specify…………………………………………………………

29. Have you undergone haemodialysis/peritoneal dialysis/blood transfusion in the last two weeks?

Yes (1) No (2)

If yes, specify and state when…………………………………………………………..

For female subjects

30. Are you expecting? Yes (1) No (2)

If yes, specify (the months)……………………………………………………………

31. How many children have you had………………………………………………..

Appendix 16 Research permit

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