THE INFLUENCE OF A NUTRITIONAL SUPPLEMENT ON LUNG FUNCTION AND IMMUNE STATUS OF HIV-POSITIVE PATIENTS IN THE MANGAUNG METROPOLITAN

Ernst Vermaak

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Promoter: Prof WMJ van den Heever-Kriek (Ph.D. Haematology and Cell Biology, UFS)

Co-Promoters: Dr. MW Brüssow (Ph.D. Physiology, UFS)
Dr. Z Hattingh (Ph.D. Nutrition, UFS)

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“Let your food be your medicine and your medicine be your food. Each one of the substances in a man’s diet, acts upon his body and changes it in some way, and upon these changes his whole life depends, whether he be in health, in sickness or convalescent. To be sure, there can be little knowledge more necessary”

Hippocrates (460-377 BC)

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This thesis is dedicated in loving memory to my parents……your dream came true.

DECLARATION

I, Ernst Vermaak, do hereby declare that this research project submitted to the Central University of Technology, Bloemfontein, Free State Province for the degree DOCTOR TECHNOLOGIAE: CLINICAL TECHNOLOGY is my own independent work. Where help was sought, it was acknowledged. Although I have followed the required conventions in referencing the thoughts, ideas and/or writing of others by means of stating the original source of information, the entire document was subjected to the search engine of the CUT in a further attempt to prevent plagiarism (search results attached-**APPENDIX A**). I further confirm that this work has not been submitted previously to any other University for the purpose to obtain a degree.

…… ……

Signature of student Date

LIST OF FIGURES AND TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

ABREVIATION	MEANING
≥	Greater/equal to
≤	Smaller/equal to
[1, 25$(OH)_2$D]	1, 25 dihydroxyvitamin D
%	Percentage
3TC	Lamivudine
µL	micro litre
A	
AI	Adequate intake
AIDS	Acquired Immune Deficiency Syndrome
ART	Anti-retroviral treatment
ARV	Antiretroviral
ATP	Adenosine triphosphate
ATS	American Thoracic Society
AZT	Zidovudine
B	
B-lymphocytes	Mature in blood stream
BMI	Body Mass Index
BTPS	Body temperature pressure saturated
C	
CD	Cluster of Differentiation
CCR3	chemokine receptor 3
CCR5	chemokine receptor 5
CDC	Centers for Disease Control
cells/mm^3	Cells counted in one cubic millimetre
cDNA	Copy deoxyribonucleic acid
$CI_{95\%}$	95% Confidence intervals
COPD	Chronic obstructive pulmonary disease
CRF	Clinical reference form
CUT	Central University of Technology, Free State
CXCR4	co-factor
Cyp A	Cyclophilin A (Also other letters of alphabet)
CYP27B1	Vitamin D-activating enzyme
D	
d4T	Stavudine
ddC	Zalcitabine
ddI	Didanosine
DNA	Deoxyribonucleic acid
E	
EDTA	Ethylenediaminetetraacetatic acid
EER	Estimated Energy Requirement
EFV	Efavirenz
ELISA	Enzyme-linked immunosorbent assay
Env	envelope gene

ABREVIATION	MEANING
ESP	Entire Study Population
ESR	Erythrocyte sedimentation rate (Viscosity)
ETOVS	Ethics Committee of the Faculty of Health Sciences, University of the Free State
F	
FEF	Forced expiratory flow
FEF_{50}	Forced mid expiratory flow (at 50% of the expired volume)
FEF_{75}	Forced end expiratory flow (at 75% of the expired volume)
FEV_1	Forced expiratory volume in one second
FFQ	Food frequency questionnaire
FIF	Forced inspiratory flow rate
FVC	Forced Vital Capacity
FEV_1/FVC	Forced expiratory volume in one second/Forced Vital Capacity ratio
Fig	Figure
FIQ	Food-intake questionnaire
FTC	Emtricitabine
G	
gag	group antigen gene
GCP	Good clinical practice
gp	Glycoprotein
g/dL	Gram per decilitre
Group M	Group Major
Group N	Group New
Group O	Group Outlier
H	
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type 1
I	
IgE	Immunoglobulin E (Also other letters of the alphabet)
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-13	Interleukin 13
IU	International units
K	
kd	kilodalton
kg/m^2	Kilogram per meter square
KJ	Kilojoules
L	
l	Litre
l/sec	Litre/second
LAV	lymphadenopathy - associated virus
LPV/r	Lopinavir/ritonavir
L/s-1	1/Liter per second
LQ – UQ	median percentage change

ABREVIATION	MEANING
M	
MAC	*Mycobacterium avium* complex
mcg	Microgram
MCH	Mean corpuscular volume
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
mg	Milligram
ml	millilitre
mm^2	millimetre/millimetre ~ millilitre
mm/h	millimeter/hour
N	
n	Number of participants
NCS	Nutrition care and support
NIAID	National Institutes of Allergy and Infectious Diseases
nm	nanometer
nm/L	nanomol per litre
NCS	Nutrition care and support
NVP	Nevirapine
P	
p<0.05	Statistical significant difference
Pathcare	Private pathology laboratory
p24	Polypeptide 24
PCP	*Pneumocystis jiroveci* (formerly *carinii*) pneumonia
PCR	Polymerase Chain Reaction
PGL	Persistent generalised
Ph	Hydrogen ion concentration
PID	Pelvic inflammatory disease
PEF	Peak expiratory air flow rate
PFT	Pulmonary function test
Pin1	Peptidal-prolyl isomerase
PLWHA	People living with HIV/AIDS
PR	Protease
Q	
QS	Quantitation standard
R	
rA	Radiographic absorptiometry
RBC	Red blood cell
RDA	Recommended Dietary Allowance
RDW	Red cell distribution width
RNA	Ribose nucleic acid
RSA	Republic of South Africa

ABREVIATION	MEANING
S	
SIV	Simian immunodeficiency virus
SDPSQ	Socio-demographic profile status questionnaire
SPG	Sub-Population Groups
Std Dev	Standard deviation
T	
T-lymphocytes	Mature in thymus
TB	*Mycobacterium tuberculosis* / Tuberculosis
Tc	T-cytotoxic
TDF	Tenofovir
Th	T-helper
THUSA	Transition and Health During Urbanisation of South Africans
TLRs	Toll-like receptors
U	
UNAIDS	United Nations Acquired Immune Deficiency Syndrome
U.S.	United States
USAID	U.S. Agency for International Development
V	
VC	Vital capacity
W	
WHO	Word Health Organisation
WCC	White cell count

SUMMARY

The HIV pandemic in South-Africa has created a new form of vulnerability for households with regards to food security and nutritional status which are vital components in the general care of HIV-infected individuals. The risk of nutritional deficiencies and malnutrition are predictors of disease progression and treatment in resource limited settings. Furthermore, HIV affects nutritional status by increasing the energy requirements, reducing food intake, affecting nutrient absorption and metabolism inadequacies due to cytokine activity and diarrhea.

Several vitamins and minerals are important in fighting HIV infection because they are required by the immune system and major organs to attack infectious pathogens. Many of these micronutrients have been found to be deficient in HIV-infected persons and several studies were launched worldwide to investigate the feasibility of food assistance and nutrient supplementation. Nutritional supplementation has been advocated in HIV-infected persons especially in low-income countries such as South Africa. Therefore, a study to evaluate the role of nutritional supplementation in HIV-positive patients becomes necessary, especially in a developing country such as South Africa. It is against this background that the present research was initiated to examine the influence of a nutritional supplement on the immune status and health status of HIV-positive/AIDS adult individuals.

The aim of the investigation was to determine if supplementation with a mixture comprised from specific minerals, vitamins and herbs over a period of one year, affected the haematological status, immune status, viral load and pulmonary function in forty (40) HIV-infected individuals living in the Mangaung Metropolitan, RSA.

A quantitative, open-labeled, before-after clinical trial was conducted at the Central University of Technology, in Bloemfontein, Free State Province in the RSA.

Socio-demographic and dietary intake questionnaires were completed. All data pertaining to anthropometric measurements, haematological status, immune status, viral load and pulmonary function were obtained my means of using standard procedures and technological equipment. The data were subjected to parametric and non-parametric statistical analysis.

The results of the present investigation show that the eating pattern of this urbanized group of individuals reflects high energy (KJ) and macronutrient intakes coinciding with sub-optimal intake of Vitamin D and iodine.

Of all the haematological variables the only statistical significant changes observed were increases in the median erythrocyte sedimentation rate (ESR) ($p=0.0219$) and mean cell haemoglobin concentration (MCHC) ($p=0.0245$) after six months of nutritional supplementation. At 12 months a statistical significant decrease in the median CD/CD8 ratio ($p<0.0048$), median Hematocrit concentration ($p<0.0312$), median mean cell volume (MCV) ($p<0.0359$), and median RDW ($p<0.0273$) accompanied a statistically significant increase in the MCHC ($p<0.0003$) at 12 months after supplementation.

At 6 months 89% ($CI_{95\%}$: 73%; 96%) of the individuals showed a decline in viral load counts with a median percentage decline of 34% ($CI_{95\%}$: 73%; 96%). At 12 months 85% [$CI_{95\%:}$ 68%; 94%] of the individuals show a decrease in viral load counts with a median percentage decline of 62.9% ($CI_{95\%}$: 50%; 78.6%) following the intake of the supplement.

The main findings of the present investigation reveal that 68% (50%-81%) of the individuals show a statistical median increase ($p=0.0302$) of 16.9% (11.5%;

36.1%) in the Peak Expiratory Flow (PEF) at six months. A significant decrease (p=0.0484) in the median FEF_{75} of 28.1% (14%; 35.3%) is observed in 70% (53%-83%) of the individuals after 12 months of exposure to the supplement. No statistical significant changes are observed for FVC, FEV_1, FEV_1/FVC and FEF_{50} over the entire trial period.

The present results suggest that a significant measurable decrease in viral load in HIV-infected individuals can be obtained by means of subjecting individuals to a nutritional fortification supplement strategy for 6 months or more.

KEY WORDS

HIV/AIDS; Nutritional supplementation; Dietary intake questionnaire; Anthropometry; Pulmonary function; Immune status; Haematological status; Liver function; Viral load.

CHAPTER 1

INTRODUCTION

1.1. INTRODUCTION

All African countries, south of the Sahara desert are known as Sub-Saharan Africa. This area represents 10% of the global population housing 67% (22 million) of the individuals infected with Human Immunodeficiency virus (HIV) (Sztam, Fawzi & Duggan, 2010; HIV and AIDS Statistics –Worldwide, 2010).

The HIV and AIDS (Acquired Immune Deficiency Syndrome) epidemic in South Africa rate amongst the highest in the world. In 2011, 10.6% (5.38 million) of the total population of South Africa was HIV-positive. The number of women living with HIV was 19.4% with 16.6% of the population between the age of 15 and 49 years during the same year and the total population of South Africa was estimated at 50 586 757 (Statistics South Africa, 2011).

The enormity of the HIV and AIDS epidemic in South Africa is further emphasized with almost one-in-three women aged 25 to 29 and 25% of men aged 30 to 34 being HIV-infected. Premature deaths attributed to HIV and AIDS have increased in the past 10 years from 39% to 75% in 2010. Children who were infected with the virus during birth were born into families where HIV and AIDS have disrupted income, health, productivity as well as the ability to care for each other. HIV and AIDS is a major contributor to poverty in South Africa as there is a 80% chance that households may lose about half their income due to the demise of the highest financial supporter (UNAIDS, 2010).

To achieve and maintain proper health, a healthy adult needs at least 500 kilocalories (2100 KJ) per day, of which 10 – 20% should comprise of protein. Unfortunately protein intake makes out a very small portion of the daily diet of many communities in Southern Africa (Spencer, Harman, Botha, Rollins, Labadarios & Visser, 2009). A study in the Ga-Rankuwa area in Gauteng, South Africa found that neither HIV-infected males nor females met the minimum prescribed daily energy requirement through their diet and indicates a serious shortcoming in micronutrient intake and dietary diversity (Venter, Gericke & Bekker, 2009). Impaired nutritional

status may be caused by a reduced intake and availability of food due to the effect of HIV and AIDS on household income (Lemke, 2005). Vitamins and minerals are organic substances that are not used to provide energy, but assist the body to utilize macronutrients to build the body. In most instances vitamins act as coenzymes. With the exception of vitamin D, small amounts of vitamin B and vitamin K, produced by intestinal bacteria, all vitamins are obtained from food or vitamin supplementation. Minerals are needed in moderate amounts and make up approximately 4% of the body weight (Marieb & Hoehan, 2010). The reduction and deficiency of vitamins A, C, E, B_6 and B_{12}, as well as zinc and selenium have been shown to compromise the immune response (Campa & Baum, 2010).

Furthermore, physiological symptoms like oral thrush; vomiting; appetite loss; reduced nutrient absorption due to diarrhea and intestinal damage may effect food consumption (Oketch, Paterson, Maunder & Rollins, 2011). The negative effect of a nutrient imbalance on the human body, especially on the immune system, is shown in malnutrition (Savino & Dardanna, 2010). Malnutrition was also recognized as a major problem since the early history of HIV infection. Even before the emergence of the HIV malnutrition has been a major cause of death due to infection (Cunningham-Rundles & Lin, 1998). Malnutrition in HIV-infected individuals is a series of conditions that occurs when the body is deprived of optimal amounts of minerals, vitamins and nutrients to maintain immune function and general health (Evans, Maskew, & Sanne, 2012). Infection and AIDS reflect on nutritional status as a “wasting syndrome” (Leah & Mascioli, 1995). Loss of body cell mass and muscle mass may be prevalent early in the course of the HIV infection suggesting that the loss is due to the HIV infection and not opportunistic co-infections (Faintuch, Soeters & Osmo, 2006).

To address the problem of poor nutrition and HIV, Gramlich and Mascioli (1995) identified several issues to be addressed: These include diagnoses of malnutrition; causes of malnutrition; specific micro- or macro elements involved; more susceptible subpopulations and the value of supplementation.

To reverse or prevent the negative effect of nutritional undernourishment in people living with HIV and AIDS, several studies were launched worldwide to investigate the feasibility of food assistance and nutrient supplementation (Oguntibeju, Van Schalkwyk, Van den Heever & Veldman, 2003; Baum, Lai, Sales, Page & Campa, 2010; Sztam *et al.,* 2010; Doa, Patel, Overton, Rhame, Pals, Johnson, Bush & Brooks, 2011; Tirivayi & Groot, 2011). A study by Venter and co-workers (2009) shows that several nutrients (macro and micronutrients) plays an important role in the prevention of malnutrition in people living with HIV and AIDS and they recommend that more studies regarding larger sample size and follow-up investigation be done.

HIV-infected individuals commonly have symptoms of airway disease associated with air trapping and significantly lower forced expiratory air flow in one second (FEV_1) and forced mid expiratory flow (FEF_{50}). Small airways disease is frequently present in HIV-positive individuals (Gelman, King, Neal, Pacht, Clanton & Diaz, 1999). With fewer opportunistic infections due to better treatment and prevention, noninfectious lung pathology like emphysema, lung cancer and pulmonary arterial hypertension have been diagnosed more frequently in people living with HIV and AIDS (PLWHA). Chronic obstructive pulmonary disease (COPD) has been associated with HIV-infection (Gelman *et al*., 1999). Experimental evidence showed that zinc supplementation may improve lung health in HIV and AIDS in developing countries (Morris & Guidhof, 2010).

1.2 PROBLEM STATEMENT

As the HIV and AIDS pandemic enters its third decade and case numbers continue to increase, no definitive cures have been found nor has an effective vaccine been developed. It is time to examine other approaches to reduce the HIV infection burden. Nutritional intervention may be one of these approaches particularly in the Free State Province of the Republic of South Africa, where the effect of a nutritional

supplement on the immune status of HIV-positive/AIDS patients has not been examined over a period of 1 year.

Malnutrition has been an endemic problem in Africa for decades, complicated by a combination of factors, and more recently the impact of HIV/AIDS. HIV infection exacerbates malnutrition through its attack on the immune system and its impact on nutrient intake, absorption and nutrient utilization. It has been demonstrated that nutritional deficiencies affect immune functions in ways that may influence viral expression and replication, further affecting HIV progression and mortality. Several vitamins and minerals are important in fighting HIV infection. They are required by the immune system and major organs to attack infectious pathogens. Several of these micronutrients have been found to be deficient in HIV-infected persons, thus nutritional supplementation has been advocated in HIV-infected persons especially in low-income countries such as South Africa.

Although the effects of HIV infection on the nutritional status are likely to be more pronounced among under-privileged populations with low dietary intake, unfortunately, relatively few data have been reported in developing countries (Ivers, Cullen, Freedberg, Block, Coates & Webb, 2009).

Data on the prevalence of malnutrition, dietary intake and/or supplementation in HIV-infected persons in industrialized countries, is widely available. However, this information is often scarce in Africa where endemic malnutrition and lack of nutrition management are common. Therefore, a study to evaluate the role of nutritional supplementation in HIV positive patients becomes necessary, especially in a developing country such as South Africa.

It is against this background that the present research was initiated to examine the influence of a nutritional supplement on the immune status and health status of HIV-positive/AIDS adult patients.

1.3 RATIONALE FOR THE RESEARCH PROJECT

The interaction between nutritional status and increased disease susceptibility is well known. Many of the immunological abnormalities seen in HIV infection are very similar to those seen in malnutrition and single nutrient deficiencies and interaction between nutrient deficiencies and HIV-induced immune deficiency has been identified (Leah & Mascioly, 1995; Ockenga, Grimble, Jonkers-Schuitema, Macallan, Melchior, J-C., Sauerwein & Schwenk, 2006).

Providing sufficient food and nutrition to meet people's needs for health, growth and development has been a long-standing challenge for African people. This challenge is further exacerbated by the emergence of HIV and AIDS. HIV contributes to malnutrition for physiological reasons related to the infection itself and also because most people living with HIV/AIDS often have diets that are deficient in energy, proteins, vitamins and other nutrients (Sztam *et al.,* 2010).

Data on the prevalence of malnutrition, dietary intake and/or supplementation in HIV-infected persons in industrialized countries, is widely available (Oguntibeju *et al.,* 2003). However, this information is often scarce in Africa where endemic malnutrition and lack of nutrition management are common. Therefore, a study to evaluate the role of nutritional supplementation in HIV positive patients becomes necessary, especially in a developing country such as South Africa.

Literature also reveals that information regarding the effect of nutritional supplementation on noninfectious lung diseases such as asthma and COPD in Sub-Saharan Africa, also seems to be lacking.

In accordance, a sound investigation should be of value if baseline dietary intake, lung function screening tests and haematological and immunological parameters be implemented to evaluate the effect of HIV infection in a specified population where malnutrition may play a role.

1.4 RESEACH AIM AND OBJECTIVES

The aim of this study was to evaluate the impact of the intake of the supplement on various and numerous variables indicative of the health status of HIV-infected individuals i.e. baseline dietary intake, immune and haematological variables.

The following immunological parameters were measured: CD4, CD8 and viral load counts.

The objectives of the study were to:

- Determine the baseline dietary intake of the study population.
- Evaluate the impact of the supplement on the immune-and health status of the patients over a period of one year.
- Determine the relationship between nutritional supplementation and pulmonary function of the study population.
- Determine the relationship between haematological parameters, immune status and lung function status.

1.5 SIGNIFICANCE OF THE RESEARCH

People living with HIV and AIDS experience nutritional deficiencies resulting in the aggravation of weight loss, decline in immunity, infectious disease and the presence of lung disease like COPD. The value of the present investigation lies within the notion that nutrient supplementation could possibly affect the HIV-status of malnourished individuals and improve quality of life as well as well being/wellness.

Not only will the results from this investigation provide constructive information to individuals infected with the HIV-virus, but also a possibility that could affect quality of life and clinical well being and/or wellness accordingly.

The present investigation could also provide information to other researchers wanting to explore this field of study.

1.6 STRUCTURE AND LAYOUT OF THE THESIS

CHAPTER 1: INTRODUCTION

A synopsis of the rationale for the research project as such and the rationale for the research design provides a benchmark for the importance and the validity of the investigation.

CHAPTER 2: **LITERATURE REVIEW**

Prelude on HIV/AIDS in general and a holistic objective comparable literature survey on nutritional intake, immunity and pulmonary function revealed in terms of the present investigation.

CHAPTER 3: METHODOLOGY

Contains a description of the methods and techniques used, as well as study procedures, sampling criteria and all other related matters on how the results are obtained.

CHAPTER 4: BASELINE DIETARY INTAKE OF HIV-INFECTED INDIVIDUALS LIVING IN THE MANGAUNG METROPOLITAN

Introduction, literature survey, methodology, results and discussion of the results revealed in this chapter.

CHAPTER 5: INFLUENCE OF NUTRITIONAL SUPPLEMENT INTAKE ON THE HAEMATOLOGICAL STATUS, IMMUNE STATUS AND VIRAL LOAD, IN HIV-INFECTED INDIVIDUALS LIVING IN THE MANGAUNG METROPOLITAN

Introduction, literature survey, methodology, results and discussion of the results revealed in this chapter.

CHAPTER 6: INFLUENCE OF NUTRITIONAL SUPPLEMENT INTAKE ON PULMONARY FUNCTION IN HIV-INFECTED INDIVIDUALS LIVING IN THE MANGAUNG METROPOLITAN.

Introduction, literature survey, methodology, results and discussion of the results revealed in this chapter.

CHAPTER 7: SUMMARY AND CONCLUSION ON THE INTEGRATED RELATIONSHIP BETWEEN THE MAIN FINDINGS

Discussion of:

- The main findings of the present investigation;
- The main findings of the present investigation relative to the findings of other researchers stated in the literature; and
- An integrated and aligned approach between the various chapters (variables) of the present investigation.

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CHAPTER 2

LITERATURE REVIEW: A PRELUDE OF HIV, NUTRITIONAL INTAKE, IMMUNITY AND PULMONARY FUNCTION IN HIV-INNFECTED INDIVIDUAL

2.1 HISTORICAL OVERVIEW

In early 1981, a rare form of cancer, Kaposi's sarcoma was detected among gay men in New York (Hymes, Green & Marcus, 1981). At the same time a rare form of pneumonia caused by *Pneumocystis carini* was also noticed in Atlanta. In June 1981 the United States Centre for Disease Control and Prevention (CDC) formed a team to investigate the occurrence of Kaposi's sarcoma and opportunistic infections. This immune compromising disease, still without a name, was officially defined as: ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS) by the CDC in September 1982. This may be described as the beginning of AIDS awareness in the world. During this time the cause of the "gay disease" was not known. It was suspected that the cause of this disease may have had an infectious origin (Noble, 2009).

The first break through in isolating the unknown virus was made in 1983, by Montagnier and co-workers at the Institute Pasteur in France. Montagnier *et al.,* investigated the so-called retrovirus and its role in the disease. They were able to culture the virus from the lymph nodes of infected individuals. After identifying the virus these researchers were able to develop an antibody test to screen for the infection. This saved the lives of many haemophiliacs as more than 100 000 had received infected blood. Retroviruses were first described in 1970 by Haward Temin and Davis Baltimore, who discovered that the virus has Ribose nucleic acid (RNA) genes unlike most viruses which have Deoxyribonucleic acid (DNA) genes. They also found an enzyme, reverse transcriptase, in the virus (Bagasra & Pace, 2008). The virus was named lymphadenopathy-associated virus (LAV), and is a virus with the CD4 cells as primary target (Conner & Kingman, 1988). In October 1983 Montagnier *et al.,* concluded that the LAV was responsible for the immune deficiency syndrome and the virus came to be known as HIV.

2.2 THE EPIDEMIOLOGY OF HIV AND AIDS

The epidemiology of HIV and AIDS is unique for every world region due to socio-demographical variables and should be discussed accordingly as subgroups are often not adequately represented (Mavedzenge, Olson, Doyle, Changalucha & Ross, 2011).

2.2.1. Global epidemiology of HIV and AIDS

It is estimated that in 2010 about 34 million adults were living with HIV and AIDS and 2.7 million people became newly infected with HIV (HIV and AIDS Statistics – Worldwide, 2010). This is 15% less than figures reported for 2001 and 21% less than in 1997 at the peak of the epidemic. The incidence of HIV has fallen in 33 countries of the world with 22 of these countries in sub-Saharan Africa (UNAIDS, 2011).

The Caribbean has the highest occurrence of HIV per capita where the mode of HIV transmission is unprotected sex. Due to increased access to treatment the mortality associated with AIDS dropped significant in this region (UNAIDS, 2011).

In South and South East Asia, with approximately 270 000 new infections during 2010 was 40% less infections that was recorded in 1996. The prevalence of HIV is lower in the South and South East Asian region than in most world areas but the size of the population causes it to present with a high HIV-infected population (4 million adults and children) (UNAIDS, 2011).

Eastern Europe and Central Asia presented with a 250% increase in the number of HIV-infected individuals from 2001 to 2010. Injection drug use and the infection of drug use partners are the main modes of HIV infection in this region. South and South East Asia are main regions where AIDS related deaths are continuing to increase (UNAIDS, 2011).

The occurrence of HIV in North America and Central Europe remains stable with an estimated 2.2 million individuals infected. AIDS related deaths remained stable since 2000, despite a 34% increase in the total number of infected individuals (UNAIDS, 2011).

The mode of transmission of new infections was: Mother to baby (11%), injecting of drugs (10%), sex between men (5-10%) and sex between men and women (approximately 66%). Of the mentioned figures, more than half were infected before the age of 25 years and died before the age of 35 years. The majority of HIV-infected people (95%) live in poor and developing countries (UNAIDS, 2008).

2.2.2. Prevalence of HIV and AIDS in sub-Saharan Africa

The sub-Saharan African area presents 10% of the global population with 67% (22 million) of HIV-infected individuals (Sztam, Fawzi & Duggan, 2010; HIV and AIDS Statistics–Worldwide, 2010). Approximately 1.9 million people were infected with HIV and 1.5 million died of AIDS in 2007 in this region. The occurrence of HIV and AIDS varies from country to country. In sub-Saharan Africa the HIV infection rate varies, with less than 0.1% in the Comoros and 26.1% in Swaziland (UNAIDS, 2008). In 2010 half the AIDS related deaths in sub-Saharan Africa occurred in Southern Africa. Furthermore 70% of all newly reported HIV infections in 2010 occurred in sub-Saharan Africa. Globally, new HIV infections peaked in 1997 with a decline in new infections as well as HIV related deaths (UNAIDS, 2011).

West and Central Africa have the lowest infection percentage amongst the younger population, with Senegal at 0.4% of the population and the Central African Republic at 5.7%. The prevalence of HIV-infected individuals in Eastern Africa varies from 1.1% of the population in Ethiopia to 4.5% in Kenya. The highest percentage of infected population occurs in Southern Africa (Mavedzenge *et al.,* 2011). The current prevalence of HIV and AIDS in the most southern region of Africa amounts to 11.3 million people (USAID, 2011). The figures in some countries neighbouring South Africa expressed as a percentage of the population are as follows: Botswana

24.8%, Swaziland 25.9% and Lesotho 23.6% (USAID, 2011). The number of people living with HIV and AIDS in South Africa where 17.8% of the population between 15 and 49 years, is amongst the highest of the 44 sub-Saharan African countries (UNAIDS, 2010).

The most vulnerable subgroup to HIV acquisition in sub-Saharan Africa is young women between 15 and 24 years. These women are eight times more likely to be infected than males of the same age due to biological susceptibility such as immature cervix. Africa is known for its gender power struggle and women do not always have choice of a sex partner or protected sex (Mavedzenge *et al.,* 2011).

2.2.3. Prevalence of HIV and AIDS in South Africa

Females represent the majority of HIV-infected persons in South Africa with about one-in-three women infected with the virus (UNAIDS, 2010). In 2006 the life expectancy of South Africans with HIV and AIDS dropped from 64 years (without AIDS) to 54 years (Dorrington, Johnson, Bradshaw & Daniel, 2006). Young adults who died from HIV and AIDS related diseases died with their offspring very young placing an increased economic burden on the family structure. This percentage of premature deaths due to HIV and AIDS increased during the last decade from 39% to 75% in 2010 (Harrison, 2009).

South Africa is divided into nine provincial regions each experiencing a different stage of the HIV and AIDS epidemic. Except for Limpopo, Eastern Cape, Western Cape and Northern Cape, the HIV and AIDS epidemic in the rest of South Africa has stabilized with the rate of new infections equaling the number of HIV and AIDS related deaths.

The prevalence and distribution of HIV-infected people in South Africa varies from province to province. The largest HIV-infected population is in KwaZulu-Natal (1.5 million, 28% of the local population) (Oketch, Paterson, Maunder & Rollins, 2011) with the Western Cape at the lower end with 3.8% of the local population being HIV-

infected (UNAIDS, 2010). In 2010 the HIV prevalence among antenatal clinic visits in KwaZulu-Natal was 39.5 % and in the Western Cape 18.5%. The percentage of HIV-infected females who visited antenatal clinics in the Free State was 30.6% (South Africa HIV & AIDS Statistics, 2011).

Dorrington and co-workers postulated that by 2015 the total population of the Free State will be 2 793 265. The growth rate will be 0.0%, total number of HIV-infected people 390 469, cumulative AIDS deaths 395 621 and about 54 381 people sick with AIDS (Dorrington *et al.,* 2006).

2.3. STRUCTURAL BIOLOGY OF HIV

The HIV virion of 120 nm in diameter (see **Figure 2.1**) belongs to the lentivirus genus and it includes the retroviruses with cone shape capsid core particles (Turner & Summers, 1999; Teixeira, Gomes, Gomes & Maurel, 2011). The icosahedral structure of the HIV contains 72 spikes formed by two viral-envelope proteins ($gp120^{env}$ and $gp41^{env}$). The lipid bilayer of the virion contains several host proteins (Class I and II histocompatibility antigens) necessary for virion budding. The nucleocapsid proteins ($p17^{gag}$, $p9^{gag}$, $p7^{gag}$ and $p24^{gag}$) making up the core of the HIV were cleaved proteolytically from a 53-kD Gag precursor. The inner part of the nucleocapsid is formed by a phosphorylated p24 polypeptide. The inner nucleotide core is formed by the p7 protein bound to the genomic RNA and a p9 protein. The two copies of the genomic RNA are involved with the enzymes protease, integrase and reverse transcriptase (Greene, 1991; Iweala, 2004; Teixeira *et al.,* 2011).

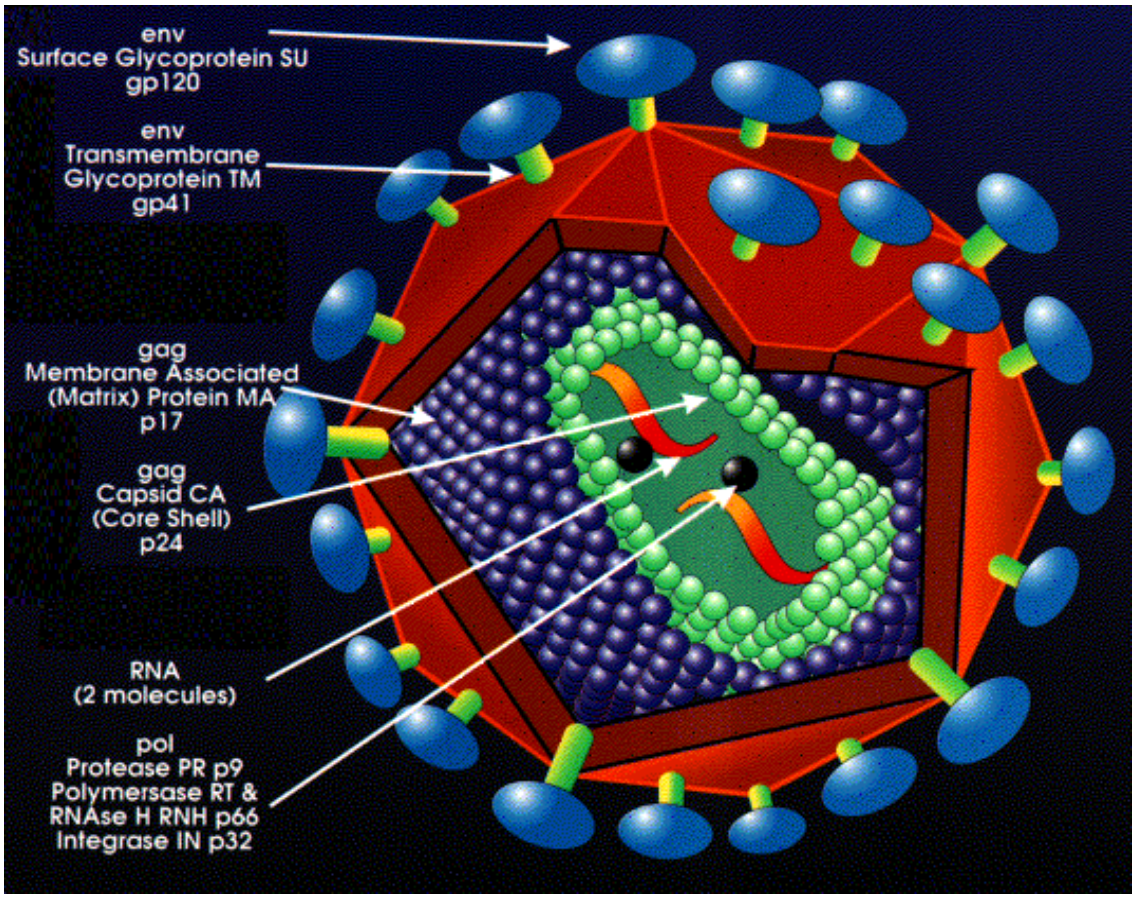

Figure 2.1. Schematic presentation of the HIV-1 Virion with cone shape capsid and core particles (Adapted from Briggs, 2008)

2.3.1. Subtypes of HIV

HIV is a virus that changes it structure easily, implying that several different strains of HIV have been identified on grounds of genetic similarities and are divided into types, groups and subtypes. There are two distinct types of HIV: HIV-1 and HIV-2 (Hahn, Shaw, De Cock & Sharp, 2000). HIV-2 is less common and is mainly, but not completely, restricted to West Africa.

HIV-1 can be classified into three groups (see **Figure 2.2**): Group M (major), group N (new, mainly in Cameroon) and group O (outlier, mainly in West to Central Africa). HIV-1, group M is divided into 11 different subtypes ranging from A to K. A combination of subtypes is named circulating recombinant forms (CRFs), for

example CRF A/C is a combination of subtype A and C (Noble, 2009). The predominant type of HIV in South Africa is HIV-1, Group M, subtype C (Redd, Avalos & Essex, 2007).

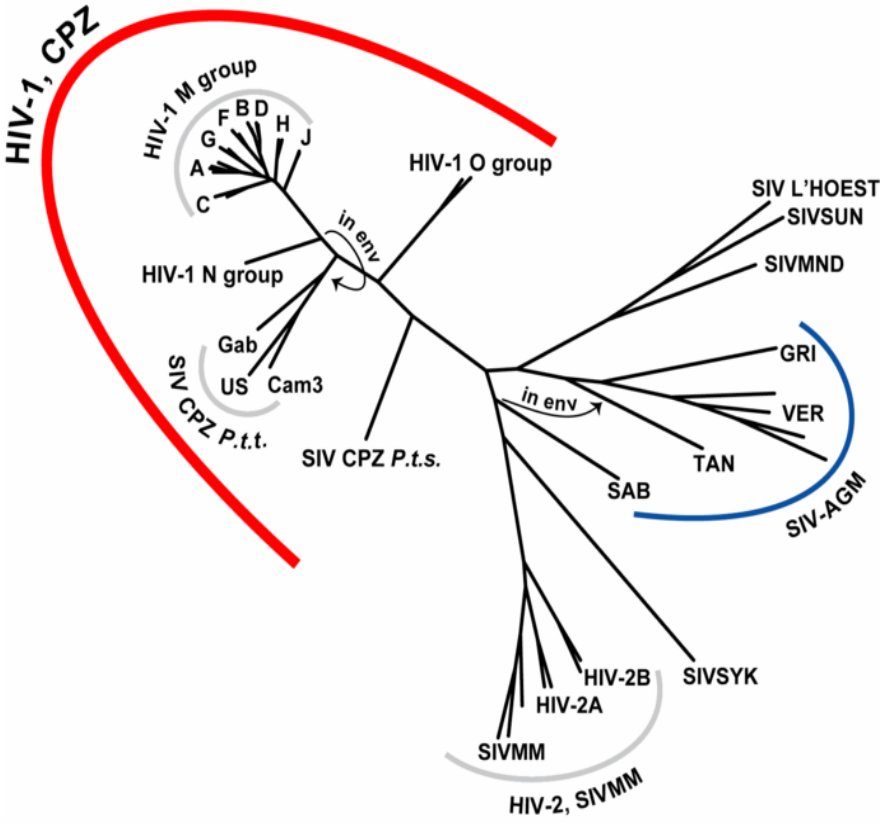

Figure 2.2. Schematic presentation of HIV-1 and HIV-2 indicating different groups and sub groups. (Adapted from Reeves & Doms, 2002)

2.3.2. Entry of HIV into receptor cells

HIV uses its gp120 and gp41 envelope protein to bind with its cellular receptor protein of the CD4 cells (see **Figure 2.3**) to enter the host cell. The gp120/gp41 connection to the CD4 cells is responsible for mediating the process of viral and cellular membrane fusion causing entrance of HIV into the host cell. The binding of gp120/gp41 to the CD4 cells and chemokine receptor CCR5 or CXCR4 (a co-factor) results in the loosening of gp120 from gp41 creating a thin structure in gp41 to penetrate into the host cell membrane, allowing membrane merging and fusion,

with entrance of HIV into the host cell (Briggs, 2008; Checkley, Luttige & Freed, 2011).

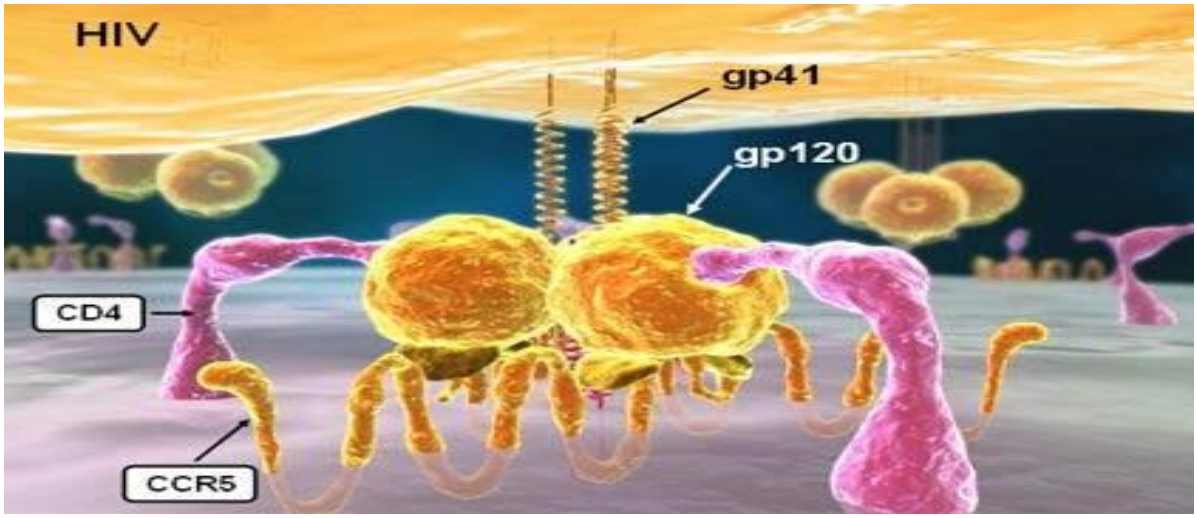

Figure 2.3. HIV cell entry demonstrating the role of gp 41 and gp 120 (Adapted from Briggs, 2008)

2.3.3 Cell entry into the host cell and replication of the HIV

Figure 2.4 is a schematic presentation of the steps taken for entry of the HIV into the host cell and the replication of the virus (NIAID, 2010):

1. Binding of the HIV to the host cell (Fusion): By means of gp120, an HIV glycoprotein with a molecular weight of 120 and co-receptors CCR5 or CXCR4 on the service of the lymphocytes or monocytes (Teixeira *et al.,* 2011). The gp120 protrudes from the virus surface and binds with the CD4 receptor of the T-cells, monocytes, macrophages and dendrite cells to facilitate the viral nucleic acid and protein to enter the host cell (Friedrich, Dziuba, Li, Endlsey, Murray & Ferguson, 2011).
2. Ribonucleic acid (RNA), reverse transcriptase and viral proteins enter the host cell (Lu, Heng & Summers, 2011).
3. Viral DNA is formed by reverse transcriptase: Reverse transcriptase is an enzyme produced by the HIV to form double stranded DNA as a copy of the viral RNA. Two RNA molecules are used for recombination during reverse transcriptase but only one DNA allele is formed (Lu *et al.,* 2011).
   Several cellular factors are necessary for reverse transcription:

- A cellular peptidal-prolyl isomerase (Pin1). Pin1 is involved with the phosphorylation of HIV-1 capsid.
- Cyclophilin A (Cyp A). Cyp A binds to the HIV-1 C89 and P90 residues tossist with virus disassembly.

4. Newly formed viral DNA forms a pre-integrated complex with the host proteins to be transported across the nucleus and integrated into the DNA of the host cell. The integration of the newly formed DNA into the host DNA is facilitated by the enzyme integrase found in HIV.
5. Translation of new viral DNA into viral proteins.
6. New viral RNA and proteins assemble on the cell surface as immature HIV forms. The immature HIV forms are converted into mature viruses by protease that cleaves the polyprotein precursors to trigger the conversion.
7. After the viruses mature, budding takes place releasing the HIV in the blood stream to initiate a new cycle of infection (Checkley *et al.,* 2011).

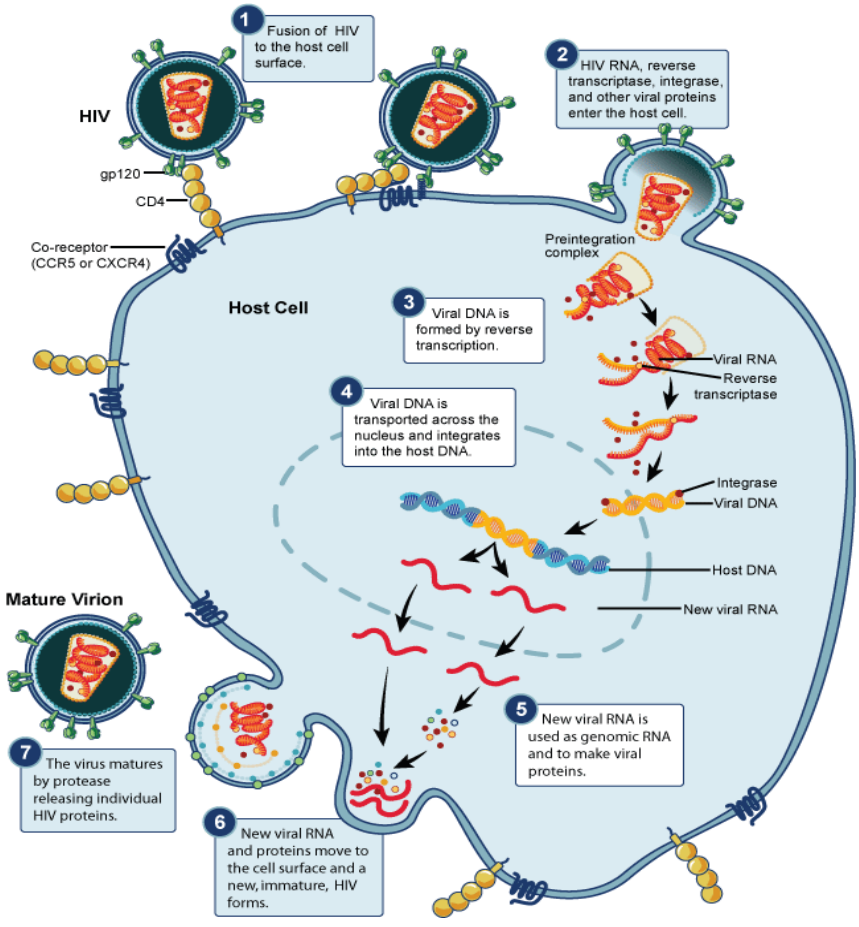

Figure 2.4. Summary of HIV entry into the host cell and the replication of the virus (Adapted from NIAID, 2010)

2.4. HIV INFECTION

This section reveals information on a classification system for HIV infection, relationship between HIV infection and TB, diagnosis of HIV and treatment of HIV.

2.4.1. Classification system for HIV infection

The revision of the classification system for HIV infection by the CDC in 1993 was to emphasize the importance of the CD4 cell count regarding the related clinical conditions found in infected individuals (CDC, 1993).

The CDC and World Health Organisation (WHO) disease classification system of HIV is primarily to compare the CD4 cell count with the presence of specific HIV related features. By using the lowest available CD4 cell count (see **Table 2.1**) and previously diagnosed HIV related conditions (**Table 2.2** and **Table 2.3**) the CDC is able to categorize HIV and AIDS (CDC and WHO, 2006; CDC and WHO, 2011).

Table 2.1. CDC Classification System for HIV-infected adults and adolescents

CD4 CELL CATAGORIES	CLINICAL CATEGORIES		
	A	B	C
	Asymptomatic, Acute HIV, or PGL	Symptomatic Conditions,#* not A or C	AIDS-Indicator Conditions*
(1) ≥500 cells/µL	A1	B1	C1
(2)200-499 cells/µl	A2	B2	C2
(3) <200 cells/µL	A3	B3	C3
Key to abbreviations: CDC = U.S. Centers for Disease Control and Prevention;			
PGL = persistent generalised lymphadenopathy.			
# For symptomatic conditions, see **Table 2.5.2**			
* For AIDS-indicator conditions, see **Table 2.5.3**			

(Adapted from CDC and WHO, 2011)

Table 2.2. CDC Classification System: Category B Symptomatic Conditions

CATAGORY B SYMPTOMATIC CONDITIONS
Category B symptomatic conditions are defined as symptomatic conditions occurring in an HIV infected adolescent or adult that meet at least 1 of the following criteria:
a). They are attributed to HIV infection or indicate a defect in cell-mediated immunity; and/or.
b). They are considered to have a clinical course or management that is complicated by HIV infection
Examples include, but are not limited to, the following:
• Bacillary angiomatosis
• Oropharyngeal candidiasis (thrush)
• Vulvovaginal candidiasis, persistent or resistant
• Pelvic inflammatory disease (PID)
• Cervical dysplasia (moderate or severe)/cervical carcinoma in situ
• Hairy leukoplakia, oral
• Idiopathic thrombocytopenic purpura
• Constitutional symptoms, such as fever (>38.5°C) or diarrhea lasting >1 month
• Peripheral neuropathy
• Herpes zoster (shingles), involving ≥2 episodes or ≥1 dermatome

Table 2.3. CDC Classification System: Category C AIDS-Indicator Conditions

CATAGORY C AIDS-INDICATOR CONDITIONS
• Bacterial pneumonia, recurrent (≥2 episodes in 12 months)
• Candidiasis of the bronchi, trachea, or lungs
• Candidiasis, esophageal
• Cervical carcinoma, invasive, confirmed by biopsy
• Coccidioidomycosis, disseminated or extrapulmonary
• Cryptococcosis, extrapulmonary
• Cryptosporidiosis, chronic intestinal (>1-month duration)
• Cytomegalovirus disease (other than liver, spleen, or nodes)
• Encephalopathy, HIV related
• Herpes simplex: chronic ulcers (>1-month duration), or bronchitis, pneumonitis, or esophagitis
• Histoplasmosis, disseminated or extrapulmonary
• Isosporiasis, chronic intestinal (>1-month duration)
• Kaposi sarcoma
• Lymphoma, Burkitt, immunoblastic, or primary central nervous system
• *Mycobacterium avium* complex (MAC) or *M kansasii*, disseminated or extrapulmonary
• *Mycobacterium tuberculosis*, pulmonary or extrapulmonary
• *Mycobacterium*, other species or unidentified species, disseminated or extrapulmonary
• *Pneumocystis jiroveci* (formerly *carinii*) pneumonia (PCP)
• Progressive multifocal leukoencephalopathy (PML)
• *Salmonella* septicemia, recurrent (nontyphoid)
• Toxoplasmosis of brain
Wasting syndrome due to HIV (involuntary weight loss >10% of baseline body weight) associated with either chronic diarrhea (≥2 loose stools per day ≥1 month) or chronic weakness and documented fever ≥1 month.

(Adapted from CDC and WHO, 2011)

In 2005 the WHO revised the clinical staging of HIV and AIDS of the case definition of HIV that was compiled in 1990. The new staging derives from clinical findings to guide the diagnoses, evaluation and management rather than a CD4 cell count. Clinical stages start with stage 1 and progress to stage 4 (advanced HIV and AIDS) (**Table 2.4** (CDC and WHO, 2006; CDC and WHO, 2011).

Table 2.4. The WHO Clinical Staging of HIV and AIDS for Adults and Adolescents

WHO CLINICAL STAGING OF HIV AND AIDS FOR ADULTS AND ADOLESCENTS
Primary HIV Infection
Asymptomatic
Acute retroviral syndrome
CLINICAL STAGE 1
Asymptomatic
Persistent generalized lymphadenopathy
CLINICAL STAGE 2
Moderate unexplained weight loss (<10% of presumed or measured body weight)
Recurrent respiratory infections (respiratory tract infections, upper respiratory infections, sinusitis, bronchitis, otitis media, pharyngitis)
Herpes zoster
Minor mucocutaneous manifestations (angular cheilitis, recurrent oral ulcerations, seborrheic dermatitis, prurigo, papular pruritic eruptions, fungal fingernail infections)
CLINICAL STAGE 3
Conditions for which a presumptive diagnosis can be made on the basis of clinical signs or simple investigations
Severe weight loss (>10% of presumed or measured body weight)
Unexplained chronic diarrhea for >1 month
Unexplained persistent fever for >1 month (intermittent or constant)
Oral candidiasis (thrush)
Oral hairy leukoplakia
Pulmonary tuberculosis within the last 2 years
Severe presumed bacterial infections (eg, pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia)
Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis
Conditions for which confirmatory diagnostic testing is necessary
Unexplained anemia (hemoglobin <8 g/dL)
Neutropenia (neutrophils <500 cells/µL)
Thrombocytopenia (platelets <50,000 cells/µL)
CLINICAL STAGE 4
Conditions for which a presumptive diagnosis can be made on the basis of clinical signs or simple investigations
HIV wasting syndrome, as defined by the CDC (see Table 2.5.3 above)
Pneumocystis jiroveci (formerly *carinii*) pneumonia
Recurrent severe or radiologic bacterial pneumonia
Chronic herpes simplex infection (oral or genital, or anorectal site) for >1 month
Esophageal candidiasis
Extrapulmonary tuberculosis
Kaposi sarcoma
Central nervous system toxoplasmosis
HIV encephalopathy
Conditions for which a confirmatory diagnostic testing is necessary
Cryptococcosis, extrapulmonary
Disseminated nontuberculosis *Mycobacteria* infection
Progressive multifocal leukoencephalopathy
Candida of the trachea, bronchi, or lungs
Cryptosporidiosis
Isosporiasis
Visceral herpes simplex infection, cytomegalovirus infection (retinitis or organ other than liver, spleen, or lymph node)
Any disseminated mycosis (eg, histoplasmosis, coccidioidomycosis, penicilliosis)
Recurrent nontyphoidal Salmonella septicemia
Lymphoma (cerebral or B-cell non-Hodgkin)
Invasive cervical carcinoma
Visceral leishmaniasis

2.4.2. HIV infection and *Mycobacterium tuberculosis* (TB)

South Africa is the country with a great burden of HIV-infected individuals and the second highest incidence of *Mycobacterium tuberculosis* (TB) infection per capita in the world (Houlihan, Mutevedzi, Lessells, Cooke, Tanser & Newell, 2010). The infection of HIV-infected individuals with TB is a serious public problem, with a much lower CD4 count in TB and HIV co-infection compared with TB infection alone (Ligidi, Gebre-Selassie & Tsegaye, 2011; Naido, Naido, Padayatchi & Karim, 2011).

The increase in TB notification also coincided with the increase in HIV and AIDS (Kapata, Chanda-Kapata, O'Grady, Schwank, Bates, Mukonka, Zumla & Mwaba, 2011). This connection between HIV and AIDS and the prevalence of TB must prompt the screening of HIV in all TB patients (Pennap, Makpa & Ogbu, 2010). The association between HIV and TB co-infection is further strengthened by the increased risk of TB infection with an increased HIV-1 RNA (Sterling, Lau, Zhang, Freeman, Bosch, Brooks, Deeks, French, Gange, Gebo, Gill, Horberg, Jacobson, Kirk, Kitahata, Klein, Martin, Rodrigues, Silverberg, Willig, Eron, Goedert, Hogg, Justice, McKaig, Napravnik, Thorne & Moore, 2011).

2.4.3. Diagnosis of HIV

The necessity for on-site diagnosis of infected HIV individuals prompted the industry to develop rapid diagnostic testing methods. On-site diagnosis improves treatment planning and it is not necessary for a trained phlebotomist to perform a venipuncture to obtain blood for a rapid test because blood is collected by means of a fingertip or heel prick with a lancet (Nabatiyan, Parpia, Elghanian & Kelso, 2011).

Standardisation of HIV-1 testing was necessary and is administrated by WHO using reference materials from the National Institute for Biological standard. The cell cultured unit standard consists of HIV-1 group M subtype B in concentration of 5.56 $\log_{10}$IU/vial. With this common standard HIV testing is a uniform test across the

world (Glaubitz, Sizmann, Simon, Hoffmann, Hesse, Lang, Kroeh, Simmler, Dewald, Haberhausen, Lindauer, Beyser, Reber, Baumeister, Wolf, Haeger & Babiel. 2011). Enzyme-linked immunosorbent assay (ELISA), Western Blot (antibody test) and Polymerase Chain Reaction (PCR) viral load testing are usually used to test and confirm HIV infection. In combination the CD4 cell count, ELISA, Western Blot and viral load are used to diagnose as well as determine the progress of AIDS and the monitoring of the anti-retroviral effectiveness. The ELISA test is a rapid screening test while the Western Blot and PCR (Viral load) are used as confirmation testing. The diagnostic tests for HIV are based on the presence of one or more of the molecules in the structure of the virus (HIV p24 antigen or HIV RNA) or the antibodies produced by the infected host (Iweala, 2004; Talha, Salminen, Sheikh, Swaminathan, Soukka, Pettersson & Khanna, 2011).

The diagnosis of HIV infection in sub-Saharan Africa is mainly done by serological rapid testing. This immuno-chromatographic test changes in less than 20 minutes colour to indicate an infected result (Santos, Clemente, Bartolo, Palladino, Cavaco, Franco, Epalanga, Pinto & Taveira, 2011).

The acute HIV infection phase follows the initial HIV infection before antibody seroconversion. During this acute phase serological rapid testing may yield false negative results and HIV RNA assay testing is recommended. Confirmation of any infected HIV results is necessary with a second specimen for HIV testing (Bassett, Chetty, Giddy, Bishop, Lu, Losina, Freedberg & Walensky, 2011). The acute phase (diagnostic window, serological latency) HIV p24 antigen and viral nucleic acid may be detected in the plasma of the infected individual. A second diagnostic window may occur if p24 antigen concentration decreases as detectable antibodies increase (Niederhauser, Strohle, Stolz, Muller & Tinguely, 2009).

2.4.4. Treatment of HIV

The treatment of HIV and AIDS includes a full range of interventions by the government of South Africa, including: Information, education, prevention

programmes, increased access to counseling and testing, the prevention of mother to child transmission, robust nutritional intervention and choice of treatment.

Treatment regimes of HIV and AIDS patients in South Africa are available in a government publication: *National antiretroviral treatment guidelines*. Newer WHO guidelines are available for the treatment of HIV and AIDS patients. Updated guidelines have been developed but will only be available in January 2012 (Telephone conversation between the author and ministerial representative E. Hadebe, 0795173333 on 27 October 2011). Some of the recommendations in the recent updates are: to discontinue stavudine as a first line treatment due to side effects of the drug. This recommendation is now implemented. Unfortunately the WHO recommendation to start antiretroviral treatment at a CD4 count below 350 cells/mm^3 was not adopted and remains at a CD4 count of ≤ 200 cells/mm^3. The new guidelines recommended earlier treatment for four groups:

- Person with WHO stage 4-disease (**Table 2.4**; WHO Clinical Staging of HIV and AIDS for Adults and Adolescents) regardless of CD4 count;
- Person with HIV and TB and a CD4 count below 350 cells/$mm^{3;}$
- Pregnant women with CD4 count below 350 cells/$mm^{3;}$ and
- Individuals with multi-drug resistance.

The European guidelines to start with Antiretroviral (ARV) treatment are also 350 cells/mm^3 (EACS Guidelines 2011). The guidelines on when to start antiretroviral treatment (ART) in HIV-infected individuals are as important as the choice of treatment. The general recommendation is to start treatment already with a CD4 cell count of 500 cells or less per micro litre (Rossouw, Richter, Martin, Avenant, Spencer, 2011).

Effective ART is usually made up of more than one drug, or even three drugs targeting different sites preventing the HIV to replicate (Coffey, 2011). Knowledge of the HIV lifecycle is used to develop treatment to inhibit viral host cell entrance, duplication and growth (HIV Lifecycle, 2011):

i) Fusion

The HIV infects specifically immune cells (T-Helper cells) carrying a CD4 molecule on its surface. The HIV enters the host cell by the binding of gp120 molecule of the virus to the CD4 receptor of the host cell. After the initial binding the CCR5 and CXCR5 proteins are activated to complete the virus fusion with the host cell. Anti-retroviral medicine that block the fusion of the HIV is called fusion inhibitors. T-20 (enfuvitide, fuzeon) binds to the HIV and inhibits the fusion process.

ii) Reverse transcription

This process takes place after HIV fusion. Inside the host cell the genetic material (RNA) of the HIV is released inside the host cell. The viral enzyme (reverse transcriptase) translates the virus RNA into DNA.

The three drugs in use to block this stage of HIV infection are:

- Nucleoside reverse transcriptase inhibitors like AZT (zidovudine);
- Non-nucleoside reverse transcriptase inhibitors like nevirapine (viramune); and
- Nucleotide reverse transcriptase inhibitors like tenofovir (Viread).

These drugs are also available in combination.

iii) Integration

After the forming of the DNA it is integrated with the DNA of the human host cell by means of a viral enzyme named integrase. This step prompts the human host cell to produce more HIV. The drug that acts on this stage of HIV infection is called integrase inhibitors, namely raltegravir (Isentress). Two potential areas to block viral duplication are with transcription (forming of messenger RNA) and translation (protein building blocks with messenger RNA).

iv) Viral assembly

Finally, the production of the HIV can be inhibited by protease inhibitors. Protease enzyme divides the protein building blocks of the HIV into smaller particles that have the ability to repeat the duplication process. These new HIV buds off from the human host cell and are set free in the blood stream (about 10.3 billion new viruses in persons not on anti-retroviral treatment).

The protease inhibitors most commonly used are ritonavir (Norvir) and tipranavir (Aptivus).

Recommended treatment regimes for HIV and AIDS in South Africa are as follows: (The South African antiretroviral treatment guidelines, 2013):

2.4.4.1. First-line therapy for adults, including pregnant women

Tenofovir (TDF) + Emtricitabine (FTC) (or Lamivudine (TC)) + Efavirenz (EFV)

2.4.4.2. Second-line therapy (Failing on a TDF-first line regimen)

Patients that continue to deteriorate virological and taking their medication correct will move on to the second–line therapy which includes zidovudine (AZT), Lamivudine (3TC) and lopinavir/ritonavir (LPV/r).

Clinical and laboratory monitoring of individuals on ARV treatment is essential to prevent drug resistance and to treat adverse events and opportunistic infections. Individuals on treatment should visit the treating doctor or nurse at 4, 8 and 12 weeks and then 3-monthly thereafter. CD4 counts and viral load have to be done 6-monthly to monitor the success of the treatment (Grimwood, 2004).

The prominence of lung disease and pulmonary infections associated with HIV infection demonstrates the severe effect that HIV has on host lung defenses of the HIV-infected individual (Beck, Rosen & Peavey, 2001). Recent studies in HIV-infected individuals show that 31% to 64% presented with pulmonary symptoms and

8% to 21% showed airway obstruction with spirometry. The pulmonary host defenses are impaired by HIV infection by means of

1) directly killing the cells directed to defenses,
2) inducing defects in the metabolic and secretory functions of effector cells,
3) shifting cell function products from immunostimulating to immunosuppressant, and
4) reducing circulating lymphocytes, monocytes and neutrophils by migration to the alveolar spaces to clear pathogens (Beck, 2005).

The direct infection of pulmonary cells by HIV alters the lung host defense because the CD4 molecule on the lymphocytes and monocytes serves as the primary cellular receptor for HIV-1, with a decrease in CD4 cell count (Beck, 2005).

HIV breaks down the immune system and promotes nutritional deficiency in infected individuals (Campa & Baum, 2010). The optimal nutritional status of HIV-infected individuals is important, because malnutrition is associated with an increased death rate (Leah & Mascioly, 1995; Ockenga *et al.,* 2006). HIV infection has a negative influence on the human metabolism even in the early stage of the infection when apparent clinical symptoms are not visible.

2.5. NUTRITION AND HIV INFECTION

To maintain optimal health a person needs energy-providing nutrients namely carbohydrates, fat and protein. In addition, water, minerals and vitamins (Spencer, Harman, Botha, Rollins, Labadarios, & Visser, 2008) play a vital role in this regard. This is even more important regarding HIV infection and AIDS as it impacts on nutritional status as a ‘wasting syndrome’ (Leah & Mascioly, 1995; Lee & Nieman, 2010). The nutritional effects of HIV infection include: An increase in resting energy expenditure (during infections), decreased food intake, depression, anorexia, malabsorption, decreased quality of life, decreased work productivity and less

dietary diversity, all contributing to the failure of gaining weight (Sztam *et al.,* 2010; Hemsworth, Hekmat & Reid, 2011). Food insecurity and increased nutritional vulnerability are major driving forces for nutritional care and support (NCS) programmes for HIV and AIDS infected individuals. The immune system requires the necessary micronutrients to maintain immunity. The immunity is compromised by HIV infection as it breaks down the immune system and promotes nutritional deficiency (Campa *et al.,* 2010).

2.5.1. Diet and nutrition in Southern Africa

To achieve and maintain proper health an adult needs at least 2100 KJ per day, of which 10% - 20% should comprise of protein. Unfortunately protein intake makes out a very small portion of the daily diet of many communities in Southern Africa (Spencer *et al*., 2008).

A study in Ga-Rankuwa in the Gauteng area, South Africa found that neither males nor females with HIV met the minimum prescribed daily energy intake through their diet and furthermore demonstrated a serious deficiency in micronutrient intake and dietary diversity (Venter, Gericke & Bekker, 2009).

A study by Lategan and co-workers in the Northern Cape, South Africa, showed that 25% of the 158 initial participants were malnourished and presented with a BMI less than 18.5 kg/m^2 with enrolment into the study (Lategan *et al.,* 2010). An investigation conducted in the Mangaung metropolitan indicated that HIV-infected women showed high energy intakes. However, a recommendation was that these individuals should reduce their fat intake and increase the intake of other forms of energy sources, such as protein (Hattingh, Walsh, Veldman & Bester, 2006).

Food for a healthy diet may represent about 30% of the total income of a family and is unaffordable for many South Africans. Interventions are necessary in South Africa to make available healthier and more affordable food (Temple & Steyn, 2011). To assess and improve the nutritional status of HIV and AIDS individuals an objective

measurement of anthropometry, clinical and biochemical status and dietary intake is necessary (Oketch *et al.,* 2011).

2.5.2. Micronutrients in HIV

Micronutrients are essential for growth, immunity and psychomotor development as they catalyze body functions and are part of specific body tissues (De Pee & Semba, 2010). Cells require minerals and vitamins in small quantities to regulate important physiological processes of the human body. (Afacan, Fjell & Hancock, 2012).

Vitamins and minerals are organic substances that are not used to provide energy but assist the body to utilise fats, protein and carbohydrates for body building. In most instances vitamins act as coenzymes. With the exception of vitamin D and small amounts of vitamin B and vitamin K, produced by intestinal bacteria, all vitamins are obtained from food or by means of vitamin supplementation. Minerals are needed in moderate amounts and make up approximately 4% of the body weight (Marieb & Hoehan, 2010).

Deficiencies of vitamins A, C, E, B_6 and B_{12}, as well as the minerals zinc and selenium have been shown to compromise the immune response (Campa *et al.,* 2010). Micronutrient deficiencies remain a major global problem, but especially in developing countries where such deficiencies contribute to impaired health and growth, neurobehavioral dysfunctions as well as defective immunity. It is also possible that certain micronutrient deficiencies may act as cofactor in HIV progression (Chen, Zhang, Li, Chen, Wei, Qu, Lui, 2011). In the Mangaung area (Free State, South Africa) HIV infection causes micronutrient deficiencies especially among HIV-infected children with glutathione, vitamin A, zinc and vitamin D being the most prominent ones (Steenkamp, Dannhauser, Walsh, Joubert & Veldman, 2009).

2.5.2.1 Vitamins

For the purpose of this study, a synopsis of specific vitamins and their respective functions relative to HIV are discussed.

Vitamin A

Vitamin A is present in the diet as all-trans retinol, beta carotene or retiyl esters. All-trans retinol is stored in the liver after esterification to retinyl ester or bounded to protein as retinol to be transported to target tissues. All-trans retinol is then oxidised intracellular to all-trans retinal by retinol dehydrogenase (Cassani, Villablanca, De Calisto, Wang & Mora, 2012).

Functions of vitamin A include maintenance of the epithelium, it acts as an antioxidant and helps to prevent damage to cell membranes (Yang, Yuan, Tao & Wang, 2011). Vitamin A deficiency will lead to increased susceptibility to lung and gastrointestinal infections, weak response to immunization and increased HIV progression (Marieb & Hoehan, 2010; Yang *et al.,* 2011 & Cassani *et al.,* 2012). The vitamin A metabolite, all-trans retinoic acid, regulates T and B cell immune responses (Cassani *et al.,* 2012).

Vitamin A supplementation reduces the incidence of measles and diarrhoea in HIV-infected children (Imdad, Sadiq, & Bhutta, 2011) as well as diarrhoea and respiratory infection in unaffected children in South Africa (Chen, Yang, Yan, 2012).

Vitamin A enhances cellular differentiation with an increase of the co- receptor CCR5 on CD4 cells that may imply that it enhances viral entry. Several trials and well documented observational studies demonstrated the benefits of vitamin A and other micronutrient supplementation (Metha *et al.,* 2010). The recommended daily allowance of vitamin A (retinol) is 0.7 to 0.9 mg per day (Spencer *et al.,* 2008).

Vitamins B Complex

For the purpose of this study, the following B vitamins will be discussed: vitamin B_1, B_2, B_3, B_6 and B_{12}.

Thiamine (B_1), riboflavin (B_2) and Niacin (B_3)

Thiamine (B_1), riboflavin (B_2) and Niacin (B_3) play important roles in the tricarboxylic acid cycle as coenzymes. This cycle is the main supplier of Adenosine triphosphate (ATP) in the human body and may be the cause of the lack of energy experienced even by asymptomatic HIV-infected individuals. Supplementation with the B group of vitamins improves the CD4 as well as the CD8 cell counts, with a longer delay and less rapid advancement of individuals to AIDS (Spencer *et al.,* 2008).

Vitamin B_6

Vitamin B_6 deficiency is well documented amongst HIV-infected individuals and is associated with neurological manifestations such as memory loss, dementia, depression and cognitive abnormalities even before the manifestation of megaloblastic macrocytic anaemia (Campa *et al.,* 2010).

Vitamin B_{12}

Vitamin B_{12} is involved in protein and carbohydrate metabolism (Sviri, Khalaila, Bayya, Linton, Stav & van Heerden, 2012). Cobalamins, vitamin B_{12} derivates, are essential for the production of methylcobalamin and adenosylcobalamin, cofactors for mitochondrial-methylmalonyl-CoA mutase and cytosolic methinine synthase (Moreira, Barsch & Yun, 2011). Vitamin B_{12} deficiencies also occur in approximately 27% of HIV-infected patients (Woods, Tang, Margo, Forrester, Jones, Hendricks, Ding & Knox, 2003).

Vitamin B_{12} is important for red blood cell formation, neurological function and DNA synthesis. Low plasma levels of Vitamin B_{12} may result rather from absorption abnormalities than inadequate intake. Vitamin B_{12} first has to be released from the

ingested food protein by gastric hydrochloric acid and gastric proteases, after which it binds to cobalophins or haptocorns in the duodenum where it is released by hydrolysis.

Free Vitamin B_{12} binds to intrinsic factor and is absorbed in the ileum. Vitamin B_{12} deficiency is prevalent in chronic conditions like Crohn's disease and HIV. Vitamin B_{12} supplementation is necessary either in the form of fortified cereal or as a supplementation (Baer & St Peter, 2011). Specific supplementation with cyanocobalamin can protect individuals against oxidative stress-associates pathologies (Moreira *et al.,* 2011).

Vitamin C

Vitamin C (ascorbic acid) is involved with the hydroxylation of carnitine, the conversion of tryptophan to neurotransmitters (serotonin and noradrenalin), the production of thyroxin and in the formation of collagen (Campa *et al.,* 2010). Vitamin C is an antioxidant, with detoxification and assists with iron absorption (Paul, 2011). Vitamin C deficiency leads to scurvy, delayed wound healing and impaired immunity (Marieb & Hoehan, 2010; Dong & Imai, 2012). Studies showed that vitamin C supplementation delayed HIV progression and the onset of AIDS (Spencer *et al.,* 2008).

Vitamin D

Vitamin D exists in several forms including 25-hydroxyvitamin D and 1,25 dihydroxyvitamin D [$1,25(OH)_2D$]. Vitamin D is also obtained by exposing the skin to sunlight to convert 7- dehydrocholesterol to cholecalciferol and vitamin D_3 (Dobnig, 2011). Ingestible forms of vitamin D are vitamin D_3 and D_2 (Beard, Bearden & Striker, 2011). Classically the main role of vitamin D has been to be essential for bone homeostasis, but recent research proves vitamin D to play an important role in modulating innate and adaptive immune function, due to the presence of vitamin D receptors in most of the immune system cells (Baeke, Takiishi, Korf, Gysemans & Mathieu, 2010). Vitamin D is involved in innate immune system regulation. Vitamin

D-activating enzyme (CYP27B1) assist in linking monocytes and pathogen sensing mechanisms (Lagishetty, Lui & Hewison, 2011).

Vitamin D, a fat soluble pre-hormone acts via metabolites like $1,25(OH)_2D$ that binds to the nuclear vitamin D receptor (Gueli, Verrusio, Linguanti, Di Maio, Martinez, Marigliano & Cacciafesta, 2012) and suppressed T-helper (Th_1) CD4 cells to enhance Th_2 CD4 cell production. Toll-like receptors (TLRs) of monocytes and macrophages play an important role in innate immune response (Lagishetty *et al.,* 2011). The stimulation of the TLRs in macrophages by anti-microbial peptides results in the conversion of vitamin D to its active form. The role of vitamin D is to assist with the induction of cathelicidin (protein with c-terminal cationic anti-microbial domain) to be released at the site of infection (Beard *et al.,* 2011; Verrusio, Linguanti, Di Maio, Martinez, Marigliano & Cacciafesta, 2012). The major function of vitamin D is associated with the absorption of calcium from the intestine and the promotion of normal bone growth and mineralisation (Gueli *et al.,* 2012).

Lower than normal serum levels of vitamin D have been found in HIV-infected individuals, although there was no correlation between vitamin D levels and CD4 cell count. It is well established that HIV treatment with protease inhibitors and non-nucleoside reverse transcriptase inhibitors interferes with vitamin D metabolism (Wasserman & Rubin, 2010). An infected correlation was found between higher vitamin D levels and decreased mortality among HIV-infected individuals (Vilamor, 2006). Vitamin D deficiency is increasing globally (Van Belle, Gysemans & Mathieu, 2011) and vitamin D supplementation should be given routinely to HIV-infected individuals for periods longer than 12 months in individuals with low serum levels (Randall, Rutstein & Shah, 2011). Vitamin D cut-off points for serum levels are >75 nm/L for sufficient, 50-70 nm/L for insufficient and, 50 nm/L for vitamin D deficiency (Makariou, Liberopoulos, Elisaf & Challa 2011; Van Schoor & Lips, 2011). According to the American Dietetic Association the Recommended Dietary Allowance (RDA) (assuming minimal sun exposure) for vitamin D should be 600 IU/day for people 1-70 years of age (Ross, Manson, Abrams, Aloia, Brannon,

Clinton, Durazo-Arvizu, Gallagher, Gallo, JonesKovacs, Mayne, Rosen & Shapses, 2011).

Vitamin E

Vitamin E is considered to be the most important chain-breaking antioxidant in body tissue and represents the first line of protection against lipid peroxidation (Pekmezci, 2011). Vitamin E is a fat-soluble antioxidant that is involved in the regulation of cell signaling and gene expression (Dong *et al.,* 2012).

Vitamin E is necessary for the functioning of the immune system by increasing the cell-mediated and humeral immune response to antigens. Vitamin E enhances macrophage phagocytic function and resistance to viral infection (Campa *et al.,* 2010). The fast proliferating immune cells are easily damaged by peroxides thus vitamin E prevents damage to the cell membranes by reducing lipid peroxidation. Supplementation with vitamin E enhances humeral as well as mediated immunity in humans (Spencer *et al.,* 2008; Marieb & Hoehan, 2010; Molano & Meydani, 2012).

Although vitamin E protects the human body against cancer, ischemic heart disease and atherosclerosis, an elevated vitamin E concentration is associated with enhancing atherosclerosis in HIV-infected individuals (Falcone, Mangili & Tang, 2010). The RDA for adults is 15 milligram of alpha tocopherol equivalents per day (Dong *et al.,* 2012).

2.5.2.2. Minerals

Minerals are involved in most of the physiological functions of the body as a component of enzymes and/or as building blocks of tissue like bone and haemoglobin (Lee & Nieman, 2010).

Zinc

Except for iron, zinc is the most abundant trace metal in the human body. An adequate intake of zinc is important for immune function and is essential for the hormone thymulin in the formation of T-lymphocytes. Zinc deficiency causes thymic atrophy, reduction in T- lymphocytes production and a reduction in macrophage activity to take up pathogens in the human body (Campa *et al.,* 2010). Zinc is required for the regeneration of new CD4 cells as well as the maintenance of T4/T8 ration and interleukin 2 levels (Metha *et al.,* 2010). Zinc deficiency leads to a reduction in circulating T-cells as well as reduced tuberculin reactivity (Gupta, Gupta, Atreja, Verma, Vishvkarma, 2009). Insufficient zinc serum levels occur in more than 50% of individuals infected with HIV (Baum, Lai, Sales, Page & Campa, 2010).

Supplementation with zinc shows a reduction in diarrhoea in HIV-infected individuals (Spencer *et al.,* 2008; Irlam, Visser, Rollins & Siegfried, 2010) as well as a reduction in respiratory disease morbidity (Imdad *et al.,* 2011). Zinc form part of the structural proteins of HIV and is necessary for effective reverse transcriptase enzyme activity (Spencer *et al.,* 2008; Paul, 2011). This mineral is involved in more than 100 enzymatic processes in the body, assists with gene expression and protein folding (Saper & Rash, 2009) and serves as co-factor for 300 known enzymes (Quintero & Guidot, 2010).

A low plasma level of zinc may occur when people consume a diet mainly consisting of maize and legumes. These food products are high in phytate that chelates the zinc in the diet (Mburu, Thurnham, Mwaniki, Muniu & Alumsas, 2010). Lower plasma zinc levels in patients with TB as a co-infection with HIV may be the result of lower a2-macroglobulin (zinc carrier protein) produced in the liver (Gupta *et al.,* 2009). The RDA for zinc is 8 mg tot 11 mg per day (Marieb & Hoehan, 2010).

Selenium

Selenium is an important component of the humeral immune repose through its role in interleukin-2 and cytokine action in the expression of T-Lymphocyte expansion (Campa *et al.,* 2010). This trace element inhibits the progression and reduces the viral load of HIV by means of its antioxidant effect through glutathione peroxidase where it is an active component. Reduced selenium status is associated with an increase of HIV disease progression and mortality (Royal & Klebert, 2009; Irlam *et al.,* 2010; Pitney, Stone, Kawai & Kupka, 2010). Selenium is a chemo preventive mediator in HIV and supplementation may increase the defense systems of the human body (Campa *et al.,* 2010) and specifically improves CD4 cell counts of HIV-infected individuals (Stone *et al.,* 2010).

Magnesium

The RDA for magnesium is 400 mg (Lee & Nieman, 2010). Magnesium is a component of certain co-enzymes in ATP and a lack of this macro mineral plays an important role in nervous system disturbances, muscle weakness and sudden cardiac death (Marieb & Hoehan 2010).

Calcium

Calcium is essential for several physiological functions in the human body. This mineral forms an important part of bone and tooth formation. Cell membrane integrity potential regulation of heart muscle contraction and clotting of blood are also influenced by this nutrient (Lee & Nieman, 2010; Marieb & Hoehan 2010).

In people living with HIV and AIDS, lifestyle, hormonal factors, decreased physical activity, lower intake of calcium and vitamin D and depression lead to lower plasma calcium levels (Borderi, Gibellini, Vescini, De Crignis, Cimatti, Biagetti & Tampellini, 2009; Campa *et al.,* 2010). Additional factors including lower BMI, lactic acidosis in addition to calcium deficiency usually lead to the loss of bone mass causing osteopenia and osteoporosis (Dobs & Brown, 2002).

2.5.3. Macronutrient in HIV

Macronutrients include carbohydrates, fats and lipids, amino acids and proteins. Carbohydrates consist of monosaccharide, disaccharide, oligosaccharide and polysaccharides. The required digestible carbohydrates in the diet should be 45% to 65% of the total calorie intake. Fats and lipids should not exceed 34% of the energy in the human diet. Protein requirement in diet is 0.8 gram of protein per kilogram of body weight. Protein intake should be 10% to 15% of the total daily energy dietary intake (Gallagher, 2012).

2.5.4. Malnutrition in HIV-infected individuals

The cause of malnutrition in HIV-infected individuals is multifaceted. Acute and chronic physiological changes associated with psychological, social and economic factors play an important role in every individual's nutritional status. These complex interactions often coexist geographically (Sztam *et al.,* 2010).

Good nutritional status of HIV-infected individuals is important as under nutrition is associated with an increased death rate (Ockenga, Grimble, Jonkers-Schuitema, Macallan, Melchior, Sauerwein & Schwenk, 2006). HIV infection has a negative influence on human metabolism even in the early stage of the infection when no apparent clinical signs are visible (Szetela & Gasiorowski, 2010). The chronic inflammation caused by infection increases the metabolic needs of the human body and may lead to the depletion of essential nutrients resulting in a malnourished person with reduced immunity. Reduced immunity is enhanced when oxidative stress becomes more prominent as nutritional reserves further decline. The immune changes in HIV-infected individuals including declines in the numbers as well as the ratio of CD4 cell count and CD8 cell count are also found in malnourished individuals due to the negative effect on the thymus gland cell proliferation. Malnutrition causes thymus atrophy affecting immature CD8 and CD4 cells contributing to impaired peripheral immune response (Savino & Dardenne, 2010). The increasing risk of death in a malnourished HIV-infected individual is independent from the viral load and CD4 cell count (Grimble, 2009).

To diagnose malnutrition, measurements such as BMI are necessary while blood analysis will help to confirm the diagnoses. Reductions in the following biochemical markers are typical of malnutrition: Albumins, haemoglobin, urea, creatinine, glucose, triglycerides, zinc, iron, selenium, vitamins A, B, D, folic acid (Szetela *et al.,* 2010) and vitamin E (Mehta, Spie, About, Giovannucci, Msamanga, Hertzmark, Mugusi, Hunter & Fawzi, 2010). Signs and symptoms of malnutrition commonly include a: BMI below 18,5 kg/m^2, cell mass loss (lean body mass) of more than 5% in three months, lack of weight gain, hormonal abnormalities and active opportunistic infections (Ockenga *et al.,* 2006).

Impaired and/or reduced nutritional status may be caused by a reduced intake and availability of food due to the effect of HIV and AIDS on the household income (Lemke, 2005; De Pee *et al.,* 2010) and physiological symptoms like oral thrush; vomiting; appetite loss; reduced nutrient absorption due to diarrhoea and intestinal damage (Oketch *et al.,* 2011).

2.5.5. Nutritional supplementation and support for individuals living with HIV and AIDS

A healthy, well balanced diet with all the necessary fluids, vitamins, minerals and other nutrients is the preferable way to supply all the body's needs. Dietary supplementation can help an individual to obtain additional micronutrients to boost the nutritional content of a diet. Supplementations are available in tablet, capsule, powder, or liquid form. Dietary supplementation may have side effects or interfere with medication and should therefore be taken with great care. According to Szetela and co-workers "Nutritional support for chronically ill patients is still perceived by medical professionals as a specialist field of expertise even though adequate supplementation of micro- and macro elements has been widely accepted as one of the key factors in successful treatment of chronic conditions like diabetes, renal, hepatic and intestinal diseases as well as HIV and AIDS. In all these cases prophylaxis of malnutrition and wasting has been proven to be cost-effective and in the case of HIV it has added value to ART alone in improving survival and quality of

life” (Szetela *et al.,* 2010). The supplementation of nutrients like vitamin A has been found to lower respiratory infections as well as AIDS related deaths (Mehta & Fawzi, 2007). A study done in the Northern Cape, South Africa, found that half the participants in the study gained significant weight after supplementation with enriched maize meal, a soy-based liquid and multivitamins. The author suggested a more aggressive supplementation programme to ensure a higher success rate in the treatment of weight gain (Lategan *et al.,* 2010). As malnutrition is a global phenomenon and not only linked to illness, mineral and vitamin supplementation and food fortification should be done according to WHO guidelines taking in consideration local conditions (Tulchinsky, 2010).

Supplementation with specific micronutrients like **vitamin D** will benefit HIV-infected individuals in particular to combat cardiovascular events (Choi, Lo, Mulligan, Schnell, Kalapus, Li, Hunt, Martin, Deeks, & Hsue, 2011) and restoring the immune homeostasis (Peelen, Knippenberg, Muris, Thewissen, Smolders, Tervaert, Hupperts & Damoiseaux, 2010). Vitamin D supplementation according to the U.S. food and drug administration should not be more than 2000 IU daily due to toxicity of this fat-soluble vitamin (Makariou *et al.,* 2011). Nutritional addition of Beta-sitosterol (naturally found in peanuts, sesame seeds and soybeans) supports the action of vitamin D to improve macrophage function. Macrophages produce cytokines and nitric oxide, an important defense mechanism against infection (Alappat, Valerio & Awad, 2010).

Oral **zinc** supplementation will reduce viral activity and enhances wound healing (Kim, Bae & Lee, 2011). **Vitamin A** supplementation in high dosage is used as a child survival intervention in HIV-infected children (De Pee *et al.,* 2010). Replenishment or additional provision of nutrients is able to enhance and restore cellular function such as phagocytosis and cytokine production of macrophages and neutrophils in individuals who suffered from mal nutrition (Afacan *et al.,* 2012).

Spirulina (*Spirulina plantensis*) has been commercially available as a food supplement for more than 10 years. Spirulina contains essential fatty acids, beta-carotene, minerals, vitamins and high quality protein. Spirulina potentiates the immune system, suppressing cancer development and viral infection (Hirahashi, Matsumoto, Hazeki, Saeki, Ui, Seya, 2002; Watanuki, Ota, Malina, Tassakka, Kato & Sakai, 2006).

Most indigenous medicinal plants are sold on informal markets and by traditional healers. Some of these plants are now commercially produced. Several products have been standardised and are commercially available including the **African potato** (Van Wyk, 2011). The African potato (*Hypoxis hemerocallidea*) also known as "magic muthi", yellow star, lotsane and inkomfe, is widely utilized by the indigenous people in Southern Africa for cancer and HIV infection, as an emetic and a tonic (Philander, 2011). The anti-cancer and anti-HIV agent in African potato is rooperol: 4, 4' diglycoside (Beta-sitosterol) (Van Wyk, Van Oudshoorn & Gericke, 1997). Beta-sitosterol and its glycoside exhibit anti-inflammatory, anti-neoplastic activities and enhance T-helper lymphocyte action to improve natural killer cell activity (Fraile, Crisci, Cordoba, Navarro, Osada & Montoya, 2012). However, clinical studies indicated bone marrow-failure and a drop in total lymphocyte count in some HIV-infected individuals using large quantities of African potato (Spencer *et al*., 2008).

2.5.6. HIV-associated 'Wasting' and nutrition

HIV wasting syndrome is defined by the CDC as: Involuntary weight loss of more than 10% of body weight; chronic diarrhoea (at least two loose stools a day for 30 days or more) or chronic weakness; Constant or intermittent fever for 30 days or more; and the absence of a condition or illness other than HIV infection that might cause symptoms (Lee & Nieman, 2010).

These symptoms are associated with clinical stage 4 of HIV infection, equivalent to AIDS (Singh & Singh, 2005). Wasting may be caused by decreased food intake,

increased nutrient requirement (hyper metabolism) and/or nutrient malabsorption. Decreased food intake may result from the loss of appetite (vomiting. nausea, altered taste, anorexia); chewing and swallowing problems (mouth and throat sores from Kaposi's sarcoma, infections like herpes, candidiasis, esophageal ulcers); decreased interest in eating (loneliness, depression); and not being able to prepare meals (poverty, weakness, AIDS induced dementia) (Dudgeon, Phillips, Carson, 2006; Lee & Nieman, 2010).

A severe increase in metabolism is also noted and may be caused by fever due to opportunistic infections and neoplastic origin. Hormone level changes such as increased cortisol levels due to chronic stress have a catabolic effect on the body by degrading skeletal muscle and slow down protein synthesis. A decrease in testosterone as well as insulin-like growth factor correlates with body mass loss and lean tissue mass loss. High levels of cytokines (proteins that produce inflammation) in Brown HIV infection affect the metabolism negatively as it causes the body to produce more fats and carbohydrates, but less protein (Dudgeon *et al.,* 2006; Slama, Le Camus, Pialoux, Capeau & Gharakhanian, 2009; Brown, 2011; Falutz, 2011).

Malabsorption of nutrients is a major contributor to wasting due to diarrhoea and inflammation of the bowel mucosa occurs in more than 50% of individuals with AIDS (Lee & Nieman, 2010).

The prevalence of osteoporosis and osteopenia is documented in HIV-infected individuals. Factors like gender, age, low body weight, sex hormones and malnutrition and calcium deficiency may contribute to bone loss. Cross-sectional studies showed a higher incidence of bone fractures amongst HIV-infected individuals (Briot, Kolta, Flandre, Boué, Ngo Van, Cohen-Codar, Norton, Delfraissy & Roux, 2011).

TB and HIV infection coincide in South Africa with more than 80% of the TB patients in KwaZulu-Natal, co-infected with HIV (Gandhi, Moll, Lalloo, Pawinski, Zeller, Moodley, Meyer & Friedland 2009). The high incidences of TB contribute to malnutrition and wasting in the HIV-infected community. The increased energy use in TB is due to an increased production of cytokines with lipolytic and protolytic activity (Gupta *et al.,* 2009).

2.6. THE IMMUNE SYSTEM AND HIV

A highly complex and interactive group of cells and cell products in the body form the immune system with its unique properties (Kubena & McMurray, 1996). Self regulation of the immune system takes place by means of helper and suppressor cells as well as close communication with other body systems including the neuro endocrine functions.

Humans are continuously confronted by intrusions from the environment, but the human immune system has evolved to render protection to the body against infectious microorganisms, which without the human being will not be able to survive (Coico, Sunshine & Benjamini, 2003; Pratt, 2003).

2.6.1 Classification of the immune system

There are two distinctive immune responses in the human body namely innate immunity and specific immune responses.

2.6.1.1 Innate immunity

Innate immunity is a natural, non-specific defense mechanism present from birth. The innate immunity consists of a variety of mechanisms such as barriers (intact skin), surface secretions, ph changes, flushing actions (urine and tears) and resident bacterial population like normal flora. The main function of innate immunity is the prevention and containment of pathogen invasion as well as the production of

antigens by antigen-presenting cells (Pratt, 2003). The innate immune system actively protects the body without prior contact against a particular invader, but it lacks specificity. Granulocytes, natural killer cells and macrophages form part of the innate immune system (Van Belle *et al*., 2011).

2.6.1.2 Specific immune responses (adaptive immune response)

The specific immune response is also known as an adaptive immune response and provides permanent protection against the known pathogen in the body. The specific immune responses are: humeral and cellular immune responses:

Humeral immunity

Humeral immunity is the result of antibodies secreted into the serum by B-lymphocytes after binding of the antigen to a specific immunoglobulin. The matured B-lymphocytes develop receptors on the surface named membrane immunoglobulin e.g. IgA and IgM. The B-lymphocytes form 5%-15% of the circulating pool of lymphocytes, the remaining 85% are T-lymphocytes and natural killer cells (neutrophils and eosinophils (Pratt, 2003). Another major element of humeral immunity is the compliment system, activated by the interaction between antigens and antibodies. This immune activation causes early apoptosis of B- and T-lymphocytes to release apoptotic microparticles in the blood stream. The compliment system is a cascade of serum enzyme reactions resulting in the lyses and then the phagocytosis of intruder antigens by polymorphonuclear cells (Coico *et al.,* 2003).

Cellular or Cell-mediated immunity

Cellular or Cell-mediated immunity is an antigen-specific immune response involving T-lymphocytes produced in the bone marrow and matured in the thymus gland. The three types of T-lymphocytes are: T-cytotoxic (Tc) CD8 cells, T-helper (Th) CD4 cells and T-suppressor cells (Coico *et al.,* 2003;

Pratt, 2003). The CD4 and CD8 are co-receptors in the periphery of the T-lymphocytes. The activation of the T-lymphocytes is by contact with antigen presenting cells like dendritic cells, macrophages and B-lymphocytes (Van Belle *et al.,* 2011). The CD8 T-lymphocytes release lytic substances, granzyme B and perforin to kill identified cells directly. The CD4 T-lymphocytes differentiate to produce interleukin, tumor necrosis factor and interferon.

2.6.2 Host Cellular Immune response to HIV infection

The early initial response of the innate and adaptive immune system occurs, but it is not in time to eliminate the HIV infection. The early immune response is responsible for the path the HIV uses to form mutations to escape detection in the human body. The presence of HIV viremia in the lymphoid tissue indicates systemic establishment of the virus in the body (Wendelsdorf, Dean, Hu, Nordone & Banks, 2011). The formation of antibodies against the gp120 (Geha & Rosen, 2008) as well as gp41 envelope is an indicator of HIV infection (Tomaras, Yates, Lui, Li, Fouda, Chavez, Decamp, Parks, Ashley, Lucas, Cohen, Eron, Hicks, Charles, Hua-Xin, Self, Landucci, Forthal, Weinhold, Keele, Hahn, Greenberg, Morris, Karim, Blattner, Montefiori, Shaw, Perelson & Haynes, 2008). The attachment sites of the HIV that lock onto the receptors of the host cells are located in the gp120 envelope glycoprotein. The cell-surface receptor on T-lymphocytes as well as other immune system cells is the CD4 glycoprotein which is the main receptor for HIV. Other co-receptors associated with CD4 molecules for HIV are chemokines (Pratt, 2003).

Figure 2.5 explains the relationship between the CD4 cell count, viral load and clinical stages of disease progression.

Initially there is a decrease in CD4 cells after infection, followed by a recovery of the CD4 count and a decrease in viral count (Crum-Cianflone, 2009; Trushin, Bren & Dabley, 2009). This initial antibody response does not provide protection against

infection, especially against infection with additional HIV strains. Eventually disease progression leads to decrease and depletion of CD4 cells with the loss of T cell function caused by the chronic persistent infection. The active CD8 cells without the presence of CD4 cells will with time loose the ability to respond to the HIV and the individual will develop AIDS (McCune, 2001; Boasso & Shearer, 2007; Green, Center & Cruikshank, 2009).

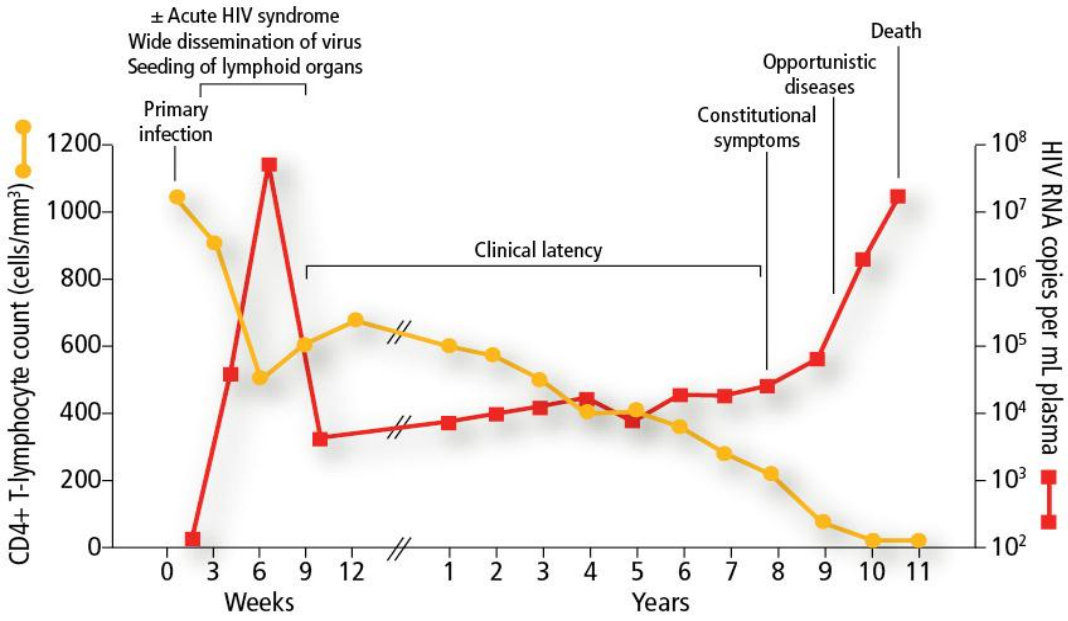

Figure 2.5. The relationship between CD4 cell count, viral load and the clinical progression of HIV syndrome (Adapted from *Living longer with HIV; new insight and issues,* 2009).

2.6.3 Nutrition and the immune system

Macro- and micronutrients affect host defenses in a number of ways (Campa & Baum, 2010):

- Cytokine and antigen receptor function can be negatively influenced by reduction of lipids and antioxidant vitamins which affect membrane structure.
- The production of antibodies may be altered by limiting amino acids and reduced protein.

- Magnesium is directly associated with antibody formation and B-cell activation. Magnesium also influences the metabolism of other metabolites such as potassium, calcium and sodium.
- Suppressed cell proliferation by limited nutrients such as zinc that modulates zinc-dependant enzymes.
- Increased humeral immunity by selenium and vitamin A supplementation.
- Increased antibody production and phagocytosis with vitamin E supplementation.
- Zinc is necessary for proper thymus function, T-lymphocyte function and T cell dependent B-cell function.

2.7 PULMONARY RESPONSE AND HIV

Respiratory and pulmonary function abnormalities remain common in HIV-infected individuals especially before the introduction of combination antiretroviral (ARV) therapy. It is also speculated that ARV medication may be responsible for irreversible airway obstruction and that obstructive lung disease is likely under diagnosed (Gingo, George, Kessinger, Lucht, Rissler, Weinman, Slivka, McMahon, Wenzel, Sciurba & Morris, 2010).

Since the first identification of HIV and AIDS, the pulmonary system has been the site mostly affected by the virus. Almost all patients develop lung complications during his/her history of HIV infection. Due to the administration of Pneumocystis prophylaxis and the introduction of highly active antiretroviral therapy (HAART) the epidemiology of lung infections has changed with a 50% decline in Pneumocystis pneumonia and bacterial pneumonia was recorded in patients treated with HAART (O'Donnell, Bader, Zibrak, Jensen & Rose, 1988; Lazarous & O'Donnell, 2007). In Africa lung infections are the most common reason for hospitalization, morbidity and mortality in people living with HIV and AIDS. Organisms such as pneumococcal pneumonia, Pneumocystis pneumonia and TB are most frequently reported (Benito, Moreno, Miro & Torres, 2011). In sub-Saharan Africa pneumonia remains the

leading cause of death and hospitalization especially amongst children (Gray & Zar, 2010). To reduce this high occurrence of respiratory infections the treatment of HAART should be accompanied by the implementation of pneumococcal vaccination (Madeddu, Laura Fiori, Stella Mura, 2010).

Pulmonary function test, particular spirometry is effective in diagnosing obstructive as well as restrictive respiratory diseases (Antwi, Gbekle, Cosmos, Ennin, Amedonu, Antwi-Boasiako, Clottey & Adzaku, 2011). HIV-infected individuals commonly have symptoms of airway disease associated with air trapping and significantly lower forced expiratory volume in one second (FEV_1) and forced mid expiratory flow (FEF 50). These results suggest that small airways disease may be present in HIV-infected persons (Gelman, King, Neal, Pacht, Clanton & Diaz, 1999). Clinical studies confirmed that emphysematous lung disease is a lung complication of HIV and AIDS (Rubin & Luca, 2011). The damage to the lung tissue may be mediated by continuous immune activation and ongoing inflammation (Coffey, 2011).

Pulmonary function testing was done in 1988 on HIV-infected individuals by O'Donnell and colleagues, who concluded that abnormally low forced expiratory flow rates frequently occurred in these individuals (O'Donnell *et al.,* 1988). Pulmonary function testing, such as the flow-volume curves indicate how the disease altered the physiological function and airflow dynamics of the lung. Measurements used to evaluate airway disease included forced expiratory volume in one second (FEV_1) and vital capacity (VC). The ratio FEV_1/VC is a parameter to demonstrate lower air flow in the lung (Takabatake, Sata, Abe, Inoue, Saito, Yuki, Shibata & Kubota, 2005; Cui, Carruthers, Mclvor, Smail, Thabane & Smieja, 2010). A decrease in the FEV_1/VC was present in HIV-infected individuals. The abnormal airway function in HIV-infected individuals may by asthma like with bronchospasm and chest tightness (O'Donnell *et al.,* 1988), alveolitis, emphysema- like pulmonary disease (Diaz, Clanton, & Pacht, 1992) and inflammatory lung disease (Boyton, Smith & Ward, 2006).

The respiratory symptoms of a HIV-infected group of smokers were compared with a similar group of HIV negative smokers. The results showed that HIV-infected individuals are extremely affected by smoking and are more at risk to develop respiratory symptoms prior to AIDS related complications. The findings were as follows: the HIV-infected group results versus the HIV negative group results: Dyspnoea (41.6% vs. 7.7%), cough (40% vs. 25%) and phlegm production (41.9% vs. 23.1%). This may demonstrate the enhanced susceptibility to lung disease of people living with HIV and AIDS (Diaz, Wewers, Pacht, Drake, Ngaraja, Clanton, 2003). Non-infective pulmonary diseases in HIV-infected individuals may include lymphocytic interstitial pneumonitis, malignancies like lymphoma and Kaposi sarcoma, interstitial lung disease. Non-infectious lung disease may present as COPD and in interstitial lung disease as restriction with decreased lung compliance (Theron, Andronikou, George, du Plessis, Goussard, Hayes, Mapukata & Gie, 2009; Hirani, Cavallazzi, Vasu, Pachinburavan, Kraft, Leiby, Short, Desimone, Squires, Weibel & Kane, 2011).

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CHAPTER 3

METHODOLOGY

3.1. RESEARCH DESIGN

The protocol design, study population, intervention (supplement), questionnaires, specific equipment and techniques used, measurements, and analysis of the data are discussed.

3.1.1. Protocol design

The trial is a quantitative, open-labeled, before-after clinical trial.

3.1.2. Research site

The present investigation was conducted in the University Clinic, Prosperitas Building, Central University of Technology, in Bloemfontein, Free State Province in the RSA.

3.1.3. Scheduled planning outlay

This investigation was divided into 2 phases each having their respective subdivisions and specified variables. These events are summarized in **Figure 3.1**.

3.1.3.1. Phase 1

During the initial phase a protocol was drafted and written approval was obtained from the Ethics Committee of the Faculty of Health Sciences, University of the Free State, Bloemfontein, before commencing with the project (ETOVS number: ETOVS 142/05).

3.1.3.2. Phase 2 (follow-up on phase 1 as specified).

Baseline screening visits were followed by monthly screening visits extended over a period of one year. The first monthly visit was scheduled seven days after the baseline screening visit. Visit two was scheduled thirty days after visit one, followed by a thirty day waiting period to the next visit for a duration of one year (final visit). Deviations of ±2 days from the scheduled visits were allowed.

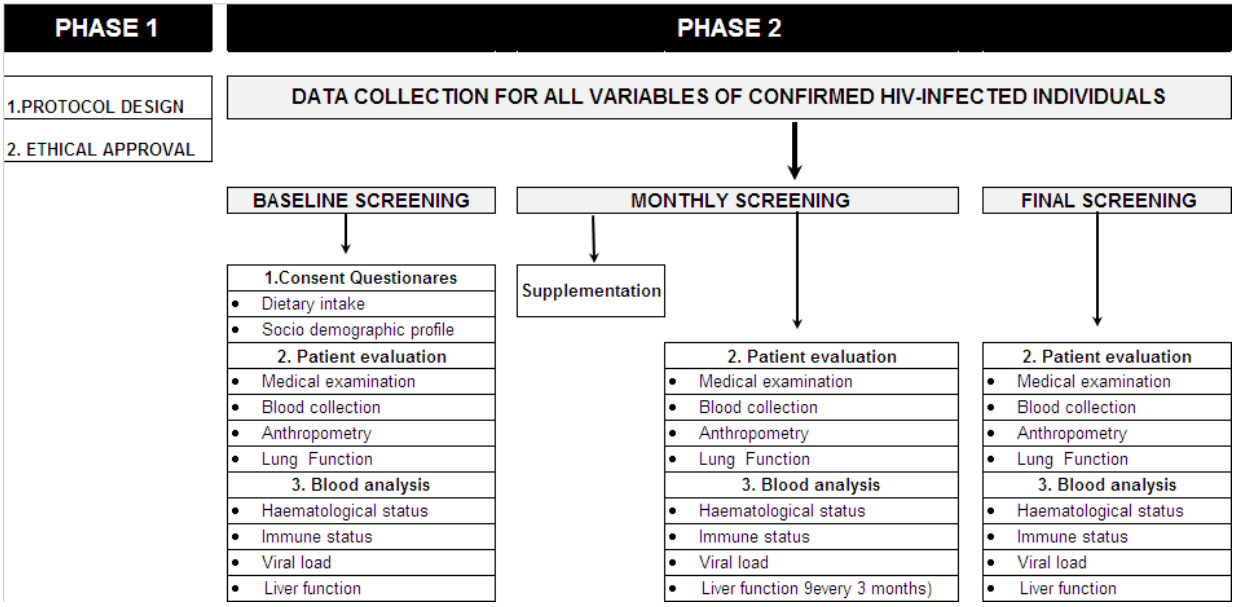

Figure 3.1. A summary of the research design and data collection of the specified variables.

3.1.4. Procedures followed during screening visits

Participants were seen at the clinic of the Central University of Technology (CUT) where specific procedures were adhered to on a monthly basis.

3.1.4.1. Procedures followed during baseline screening

Participants signed the consent form at their first visit after being informed.

A registered dietician, researcher and local language interpreters interviewed the participants to complete the socio-demographic profile status questionnaire (SDPSQ) as well as a validated food-intake questionnaire (FIQ), (See 3.4).
Physical and medical evaluations were performed on the participants by registered medical personnel to evaluate the health status of all participants.

The researcher gathered information on bodyweight, height, as well as hip and waist circumferences to calculate anthropometric data (to calculate the BMI) by means of standard procedures (Lee & Nieman, 2010) and performed pulmonary function tests by means of spirometry (See 3.5.2.6).

Specialized medical personal took blood samples from all participants during each and every screening visit (see 3.5.2.2.) to gather information on haematological status and liver functions (3.5.2.3), immune status (see 3.5.2.4), and viral load (see 3.5.2.5). Pathcare (private pathology laboratory) performed the laboratory analysis on all of these biochemical variables: The analytical methods applied are described in section 3.5.2: Laboratory investigations.

3.1.4.2. Procedures followed during monthly screening

The participants visited the clinic at the CUT on a monthly basis and all data was gathered by following the same procedures as outlined above (see 3.4.2.1.). Viral load and liver functions, however, were determined every 3 months during the follow-up screenings including the final screening.

Compliance of the participants to adhere to the protocol and supplement intake was monitored throughout the trial (see 3.3.3 and 3.7.2).

3.2. STUDY POPULATION

The participants were HIV-positive volunteers living in the greater Mangaung Metropolitan. Recruitment was done by registered medical personnel, by word of mouth, transpiring in a snow-ball sampling effect. Both females and males in the age group of 18 to 65 years were included. None of the participants were on anti-retroviral therapy or any form of chronic medication (Clinical category A 2).

The baseline CD4/CD8-cell counts, viral load count and haematological data of the included participants were used as internal controls as no separate ‘control’ group took part in this investigation because:

- The immunological and nutritional status of people living with HIV and AIDS is different from those who are HIV-negative. It is believed that the response from HIV-negative people after supplementation may be very poor, thus

supplementation may not reflect a true influence of the nutritional supplement on the immune status of the HIV-negative group.

- It is not ethical to use a HIV-positive control group, as they then would have been excluded from the use of medication (ART), treatment or discrimination in terms of gaining access to the possible benefits of the supplements.

In accordance, all participants served as internal controls.

3.2.1 Sample size

A total of 132 participants pitched up for the initial baseline screening tests conducted over a period of 5 years (2006-2010). Participants who dropped-out due to withdrawal (see 3.2.2.3) or who did not comply with the terms and/or conditions set out in the research design, were replaced with individuals who met the inclusion-exclusion criteria(see 3.2.2) in an attempt to raise the bar in terms of increasing the 'power of the statistical outcomes'. The final number of participants that met all requirements at 12 months was forty (40).

3.2.2. Inclusion, exclusion and withdrawal criteria

Strict inclusion, exclusion and withdrawal criteria ensured a homogeneous participant population with as little variation as possible:

3.2.2.1. Inclusion criteria

- HIV-positive male and female participants aged 18 to 65 years;
- CD4-cell counts between 200–500 cells/mm^3;
- Medical history, physical evaluation, and laboratory results within the range of clinical acceptability, acknowledged by the clinical investigator;
- Agreement to undergo a pre-study physical evaluation and pre- and post-study laboratory investigations; and
- Ability to understand and willingness to sign the agreement of informed consent.

3.2.2.2. Exclusion criteria

- History of psychiatric disorder, antagonistic personality, poor motivation to participate in this study or limited ability to comply with protocol requirements;
- History of, or current compulsive alcohol abuse (>10 drinks weekly), or regular exposure to other substances of abuse;
- Participation in another study with an experimental drug within 10 weeks before the first administration of study supplement and/or screening visit;
- History of hypersensitivity to the supplement;
- Loss of blood equal to or exceeding 500 ml during the 10 weeks before the administration of study medication;
- Heavy smoking (more than 20 cigarettes per day);
- Pregnant women;
- Diabetic HIV-positive participants; and
- On anti-retroviral therapy or any treatment for chronic diseases.

3.2.2.3. Withdrawal criteria

Participants had the right to withdraw from the study at any time, irrespective of the reason, without detriment of their medical care. These participants were handled as drop-outs. Participants who dropped-out were replaced with individuals who met the inclusion criteria. The study had 92 drop-outs because of withdrawal or non-compliance.

3.2.3. Participant identification

The following strategy for identification purposes during follow-up screening visits was implemented and adhered to:

- Each enrolled participant received a specific number and retained this number throughout the trial.

- Each enrolled participant retained his/her initials obtained from a copy of his identification book (three digits); and
- Each enrolled participant was identified by date of birth (six digits).

3.2.4. **Participant's informed consent** (see **APPENDIX B**)

Before commencement of any of the screening procedures, the participants were given written notice and were also verbally informed by the researcher (with the help of language interpreters) of the nature, purpose and possible risks involved in the screening procedures as well as the purpose, procedures, restrictions, obligations, remuneration, insurance coverage and possible adverse supplement reaction relevant to the study.

The participant information sheets and informed consent forms were available in English, Afrikaans and the language most often spoke in the area (Sotho). Each participant was provided with the participant information sheet and informed consent form in the language of his/her choice and retained copies of the participant information sheet and signed statement of informed consent.

Both the informed consent discussion and the participant's written information were included to provide adequate information for the participants to understand and the participants in turn voluntarily accepted the terms of the study, and agreed to co-operate in its conduct by signing the dated informed consent form.

3.2.5. Restrictions on participants

The following conditions prevailed:

3.2.5.1. Medicines

All concomitant medication was documented in clinical reference form (CRF) and the researcher notified if any additional medication was required during the study period.

3.2.5.2. Diet

The participants continued with their normal diet similar to their baseline diet.

3.2.6. Safety of the participants

During all screening tests physical and medical evaluations (see 3.1.3. and 3.1.4) were adhered to in a professional and ethical manner (see 3.7).

The supplement was ethically approved and is comprised of micronutrients and herbs only and contains no substances other than those specified substances (see 3.3).

The supplement was not used for purposes other than as directed in terms of the approved protocol.

3.2.7. Financial implication for the participants

There were no financial implications for the participants. The participants did not have to pay for their medical consultations or any of their clinical results. They received the treatment and supplement free of charge. The participants did not receive any remuneration for their participation in this study.

3.3. SUPPLEMENT

This section provides information on record keeping (inventory), formulation, and directions for use of the product (Bermins product).

3.3.1. Inventory

Administrative records were kept on whom, and when, a participant received the unit-package containing the supplement. On completion of the study, the remaining units (with exception of the retention samples) were returned to the sponsor. The retention samples were stored for a period of time in accordance with the sponsor's storage instructions.

3.3.2. Product formulation

The product contains the following ingredients and quantities (**Table 3.1**).

Table 3.1. Composition of the supplement

SUBSTANCE	QUANTITY
Beta sitosterol (Hypoxis hemerocallidrea*)	40 mg
Biocidin	100 mg
Colostrum	100 mg
Spirulina	100 mg
Beta carotene	2 mg
Tocoferol (E)	15 iu
Nicotinamide	10 mg
Pyridoxine (B6)	12 mg
Ascorbic acid (c)	50 mg
Thiamine (B1)	75 mg
Riboflavine (B2)	75 mg
Cyanocabalamin (B12)	0,025 mg
Folic acid	0.6 mg
Calcium D - Pantotenate	15 mg
Biotin	0.05 mg
Zinc ACC 10%	15 mg
Selenium oxide 2%	5 mg
Magnesium oxide	25 mg
Calcium carbonate	450 mg

*(Van Wyk, Van Oudshoorn & Gericke, 1997).

3.3.3. Directions for use

The participants took one (1) tablet of the supplement once daily early in the morning and continued with their normal diet of the day. This procedure was followed throughout. Red Cross workers visited the participants on occasion in the afternoons to monitor that the number of pills were taken as prescribed.

3.3.4. Supply, storage and dispensing

A suitable number of units (plastic bottle containing 31 pills) to be used by the participants and for retention sample purposes were provided by the sponsor in bulk. The units were stored according to the storage instructions for the product in a confined enclosure at the institution (CUT). On clinic days and adhering to the administration procedures and purposes (see 3.1.3), each and every product-unit was labelled for each and every participant accordingly (see 3.2.2) i.e. the

participant number on the label corresponded to the number allocated for each participant.

3.4. QUESTIONAIRES

Two questionnaires namely a socio-demographic status questionnaire and a Dietary intake questionnaire were completed only at baseline. Local language interpreters were available to help avoid misunderstanding during the completion of the questionnaires.

3.4.1. Socio-demographic status questionnaire (See **APPENDIX C**).

Socio-demographic details including age, gender and residential area were obtained from each participant by means of a one-on-one interview to complete the questionnaire. The following information was collected by the researcher: Financial and employment status, level of education, marital status, monthly income, type of house, amount spent weekly or monthly on feeding, available cooking facilities, smoking habit, the number of children and the number of persons living in the house.

3.4.2. Dietary intake

At first visit a validated quantitative food frequency questionnaire (adapted from the Transition and Health During Urbanisation of South Africans (THUSA) study (Potchefstroom University) with a reliability of 90% was used to gather information on the habitual types and quantities of food and drink intakes by participants to determine the habitual intakes in terms of total energy, macronutrients and micronutrients (see **APPENDIX D**).

The food frequency questionnaire (FFQ) was completed by a registered dietician appointed on a part time basis.

Both traditional and western foods were included in the food frequency questionnaire.

Explanations were used to assist with the accuracy of the size of food portions described by the participants. Each participant was asked to demonstrate the quantity of a given food that he or she consumed on a daily, weekly or monthly basis. Local language interpreters were available to help avoid misunderstanding during the completion of the questionnaires.

The portion sizes were estimated using house hold measures and converted to grams using the conversion figures in the Medical Research Council of South Africa Food Quantities Manual (Langenhoven, Kruger, Gouws & Faber, 1998). The quantities of food consumed on a daily basis were entered accordingly. The quantities of food stuff selected by the participants per day was calculated as food in grams consumed per week divided by 7 days or food in grams consumed per month divided by 30 days. The recorded food items were coded by means of food composition tables of the Medical Research Council of South Africa (Langenhoven *et al.,* 1998).

Complex dishes not appearing in the food composition tables were broken down into individual ingredients and weights and coded as such. The dietary data were analysed by means of a computer software programme, FoodFinder 3® (Medical Research Council, South Africa; June 2012; License Ernst Vermaak: C5997A8F) by a registered dietician. The energy intake was compared to the estimated energy requirement. Macro and micronutrient intake was compared with the recommended daily allowances (RDA) or Adequate Intake (AI). A value of <67% of the RDA/AI was considered to be inadequate for the individuals.

3.5. MEASUREMENT INSTRUMENTS AND TECHNIQUES

This section describes the kinds and types of physical and laboratory measurements made.

3.5.1. Anthropometry

Anthropometry involves obtaining physical measurements of an individual, and relating these measurements to standards that also reflect their health and nutritional status such as malnutrition (Lee & Nieman, 2010). The following anthropometric data was obtained (see **APPENDIX E)**:

Height

Measured in centimetre with Seca, wall mounted stadiometer able to measure to the nearest 0.01 centimetres. The stadiometer is fitted with a right-angle headboard that can be moved up and down from a height of one meter to two meters. The stature or standing height of the participants was measured as follow: Bare feet flat on the floor and heels together against the wall. Arms at side of body with shoulders relaxed, head, shoulders and buttocks against the wall and the headboard resting on the crown of the head. The observer's eyes must be level with the headboard to avoid parallax errors (Lee & Nieman, 2010).

Weight

Weight was measured in kilograms with a digital Tanita BWB 800 scale (able to measure 100g to 200kg). The scale was placed on a level, flat and hard surface and the scale was set to zero before the participant step onto the scale. The participant had to stand in the middle of the scale platform with weight equally distributed on both feet without touching anything (Lee & Nieman, 2010).

Body mass index (BMI)

Body mass and height were used to calculate the BMI:

$$\textbf{BMI}\ (kg/m^{2)} = \frac{\text{Mass (kg)}}{\text{Height (m)}^2}$$

The following BMI cut-off points were used (Lee & Nieman, 2010):

Underweight	< 18.5
Normal weight	=18.5–24.9
Overweight	> 25–29.9
Obesity	≥ 25–29.9

Waist and hip circumferences

Waist and hip circumferences were measured with a non-elastic, flexible tape with accuracy to the nearest 0.1 centimetre. The waist circumference was measured halfway between the lower rib line and iliac crest and the hip circumference on the iliac crest line (Lee & Nieman, 2010).

During all of the measurement sessions, each participant stood with relaxed, arms comfortably placed to the side and feet held together, however, some measurements required the participants to place their feet apart. The participants presented themselves in minimal clothing to allow measurements to be done correctly and efficiently. The measurements were taken according to standard procedures (Lee & Nieman, 2010).

Two trained researchers at a time did the measuring i.e. one measured and the second researcher noted the measurement. This ensured accuracy of site location, correct sequence of measurement sites and accurate reading. The recorder repeated the value as it was being recorded in order to enable the measurer to do an immediate check. The measurements were repeated 3 times at each site on each participant and the average value calculated and noted.

3.5.2. Patient evaluations

Laboratory investigations were limited to physical evaluation, blood sample analysis and pulmonary function testing.

3.5.2.1. Medical evaluation

A general physical evaluation was performed on each and every participant by a clinical physician during all monthly visits. All concomitant medications given to participants during the course of the study were documented in a clinical reference form (CRF) and specified for each and every individual.

3.5.2.2. Blood sample collection

Blood samples were collected during the screening and monthly visits by registered medical personnel and transported to Pathcare (Pathology laboratory) for the analysis of the immune status, liver functions (health status), and haematological parameters as outlined in **Figure 3.1**.

3.5.2.3. Pulmonary function tests

The methods, standards and considerations for the pulmonary function testing were adopted from the American Thoracic Society's (ATS) task force document published in the *European Respiratory Journal.* This is to ensure that pulmonary function testing comply with the recently established international standards (Miller, Hankinson, Brusasco, Casaburi, Coates, Carpo, Enright, Van der Grinten, Gustafsson, Jensen, Johnson, MacIntyre, McKay, Navajas, Pedersen, Pellegrino, Viegi & Wanger, 2005).

3.5.2.3.1. Spirometry

Spirometry (meaning the measuring of breath) is the most common of the pulmonary function tests (PFTs), measuring lung function, specifically the amount (volume) in relation with the rate of air flow that can be maximally exhaled following maximum inhalation.

The spirometry test is performed using a device called a spirometer which graphically displays a flow-volume loop (spirogram), which graphically depicts the rate of airflow on the Y-axis and the total volume inspired or expired on the X-axis (see **Figure 3.2**).

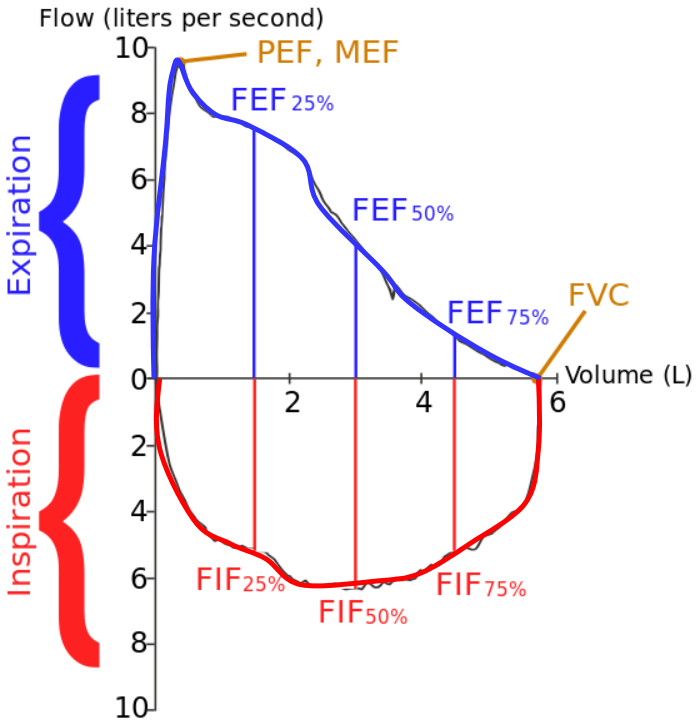

Figure 3.2. Adapted graphic illustration of a flow-volume loop depicting the relationship between rate of air flow on the Y-axis and the volume of air expired on the X-axis. (Adapted from Ruppel, G.L. 2003).

Specific values for various and numerous specified parameters (as indicated in the spirogram) are also automatically calculated by the computerized software installed in the portable Jaeger lung function machine. The following pulmonary function variables are automatically calculated:

- The forced vital capacity (FVC);
- Forced expiratory volume in 0.5, 1.0 and 3.0 seconds ($FEV_{0.5,\ 1.0\ and\ 3.0}$);
- The FEV_1 / FVC expressed as a percentage (FEV_1 / FVC X 100);
- The peak expiratory air flow rate (PEF);
- Forced expiratory flow rate at 25%, 50% and 75% of the expired volume ($FEF_{25\%,\ 50\%\ and\ 75\%}$); and

- Forced inspiratory flow rate at 25%, 50% and 75% of the inspired volume ($FIF_{25\%,50\% \text{ and } 75\%}$).

The highest rate of flow (exhale / blow) is effort dependent and occurs at 25% of the expired volume. The flow-rate in the smaller airway is less effort dependent and is influenced by the elastic recoil of the lung and the integrity of the smaller airways.

In terms of these variables a diagnosis regarding pulmonary function can be made. However, the flow-volume curve only gives a clear indication on how a specific type of disease has changed the shape, flow and volume values of the pulmonary system during maximal expiration following a maximal inspiration. To gain information on lung disease, the type of disease still have to be confirmed by means of a history of the participant, physical evaluation and other evaluations like radiography, bronchoscopy, microbiology and cytology.

3.5.2.3.2. Requirements for the equipment

The Spirometer must be capable of accumulating volume for ≥15 s and measuring volumes of ≥8 L (BTPS) with an accuracy of at least ±3% of reading or ±0.050 L, whichever is greater, with flows between 0 and 14 L/s-1. The total resistance to airflow at 14.0 L/s-1 must be ‹1.5 cmH2O/L-1/s-1 (0.15 kPa/L-1/s-1). The total resistance was measured with a tubing, valves, pre-filter, inserted between the participant and the Spiro meter. The filter is important for this study to prevent cross contamination of organisms that may infect participants performing a lung function test.

The volume–time curve was plotted as hardcopy, the volume scale ≥10 mm/L-1 (BTPS). For a screen display, 5 mm/L-1 is satisfactory. The time scale was ≥20 mm/s-1. When the volume–time plot was used in conjunction with a flow–volume curve, the time scale requirement was reduced to 10 mm/s-1” (Miller *et al.,* 2005).

3.5.2.3.3. Quality control

Calibration is a procedure for establishing the relationship between sensor-determined values of flow and volume and the actual flow or volume to ensure reliable lung function results. The Spirometer is calibrated with a calibration syringe. The syringe discharged at least three times with a variation between the volumes less than 3% (Miller *et al.,* 2005).

3.5.2.3.4. Test procedure

After the setup of the equipment and a quality check, the technologist instructs and demonstrates the test procedure to the participant. The maneuver consists of three distinct actions: 1) Deep and maximal inspiration; 2) a forced and maximal expiration performed like a hard blow of air; 3) continuation of exhalation till the air flow is zero for two seconds at least for six seconds (Enright, 2003).

The participant was encouraged to do better after every effort by demonstration and exaggeration. At least three efforts are needed of which the sum of the forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) of the three efforts do not vary more than five percent (5%) (Miller *et al.,* 2005).

3.5.3. Blood sample analysis

Blood sample analysis and pulmonary function tests were performed.

3.5.3.1. Haematological variables

A Beckman Coulter machine was used to obtain information on the following haematological variables:

- Red cell count;
- Haemoglobin;
- White cell count;
- Differential count;
- Hematocrit (packed cell volume);

- Mean cell volume (MCV);
- Mean cell haemoglobin (MCH);
- Mean cell haemoglobin concentration (MCHC);
- Red cell distribution width (RDW), and
- Platelet count.

3.5.3.2. Immunological variables

Purpose: To determine the immunological status of the participant: The CD4/CD8-cell counts are essential measures of risk of contracting opportunistic infections and thus used as an indicator for instituting disease prophylaxis:

Methodology of testing implemented

The analysis was done by a pathology laboratory in Bloemfontein using the BD *FACSCalibur*^{TM} flow pyrometer for cell enumeration (BD Biosience, 2350 Qume Drive, San Jose, CA 95131, USA). A BD Tritest^{TM} reagent kid was used for the CD4 and CD8 cell counts. The immunofluorescent labelling of the whole blood lymphocytes using BD Tritest ^{TM} reagents follows:

- The sodium citrate tube containing the peripheral whole blood sample of a participant was mixed for a few seconds on a vortex mixer to allow for proper mixing of the sample;
- The sample was checked for clots with applicator stick and the applicator stick subsequently safely disposed;
- 20 µL of BD CD3/CD4/CD45 reagent was placed into a Trucount tube;
- 50 µL of participants' whole blood was added to the tube containing the BD reagent;
- The same procedure was repeated with the Immuno-Trol control;
- The tube was vortexed gently and incubated in the dark at room for 15 minutes at 20-25^{o}C;
- 450 µL of IX BD FACS lysing solution was added to the mixture of antibody and blood and was vortex gently again;

- The tube was further incubated in the dark at room temperature for 15 minutes; and
- The specimen mixture was then analyzed with a flow cytometer. The stained lysed sample was stable for five (5) days.

The CD4/CD8-cell counts were measured using a principal method called flow cytometry where blood cells are passed through a specially designed flow chambers and the physical characteristics of the cells (size and granularity) were measured with laser technology together with the detection of any surface markers such as CD4 which have been stained with fluorescent monoclonal antibodies. The four-colour direct immunofluorescence technique with a suitably equipped flow cytometer for CD3, CD4 and CD8-cell counts were used for this study supplied by Becton Dickinson (BD Ltd, South Africa).

Sensitivity:

CD4 cells are measured relatively accurately when their number exceeds 50 cells/ml but precautions prevail: Since CD4 cell counts can fluctuate due to extraneous factors, it should always be correlated with the CD4 cell% measurement. True increases/decreases in cell% were accompanied by corresponding increases/decreases in cell%. Single out-of-range results should be confirmed by re-testing.

3.5.3.3. Liver functions

An unicel DXC 880i Synchron access clinical system was used to obtain measurements that reflect liver functions (see **APPENDIX F**). These tests were done every 3 months to test toxicity of the supplement. In accordance, no results are shown and no statistical analysis on any of the numerous variables were analised statistically.

3.5.3.4. Viral load

Purpose: Quantification of HIV count. This is the test for staging HIV disease and/or monitoring the efficacy of treatment.

Methodology of testing

The Amplicor HIV-1 Monitor Test, v1.5, is used with plasma specimens only. Five (5) ml of blood was collected from each individual into a sterile EDTA sample tube.

The plasma was separated from the whole blood by centrifugation at 800 revolutions per minute for 20 minutes at room temperature. The separation took place within 6 hours after collection. Plasma specimens were stored at -80^0C before analyses.

The Amplicor HIV-1 Monitor Test, v1.5 may be used with either of two specimen preparation procedures, the standard procedure or the Ultra Sensitive procedure. The standard procedure was used in the present investigation. In the standard specimen preparation procedure, HIV-1 RNA was isolated directly from plasma by lysis of virus particles with a chaotropic agent followed by precipitation of the RNA with alcohol. A known number of quantitation standard RNA molecules were introduced into each specimen with the lysis reagent. The HIV-1 quantitation standard was carried through the specimen preparation, reverse transcription, amplification and detection steps and used for the quantitation of HIV-1 RNA in the test specimen.

The Amplicor HIV-1 Monitor Test, v1.5 is based on five major processes:

i. Specimen preparation;
ii. Reverse transcription of target RNA to generate complementary DNA (cDNA);
iii. PCR amplification of target cDNA using HIV-1 specific complementary primers;

iv. Hybridization of amplified DNA to oligonucleic probes specific to the targets; and
v. Detection of the probes bound amplified DNA by colorimetric determination.

In the Amplicor HIV-1 Monitor Test, v1.5, the reverse transcription and amplification of HIV-1 and quantitation standard (QS) RNA occur simultaneously. The Master mix reagent contains a biotinylated primer pair specific for HIV-1 and QS target nucleic acid.

The quantization of HIV-1 viral RNA was performed using the HIV-1 quantization standard (QS) (Tris HCL buffer: <0.001%, non-infectious in vitro transcribed RNA; microbial containing HIV-1 primer binding sequences) and a unique probe binding region (<0.005% Poly rA (synthetic), EDTA, Amaranth dye, 0.05% Sodium azide). The HIV-1 quantitation standard is a non-infectious RNA transcript that contains the identical primer binding sites as the HIV RNA target and a unique probe binding region that allows quantization standard amplicon to be distinguished from HIV-1 amplicon. The quantization standard was incorporated into each individual specimen at a known copy number and is carried through the specimen preparation, reverse transcription, PCR amplification, hybridization and detection steps along with the HIV-1 target and was amplified together with the HIV-1 target. HIV-1 RNA levels in the test specimens was determined by comparing the HIV-1 signal to the quantization standard signal for each specimen. The quantization standard compensates for effects of inhibition and controls for the amplification process to allow the accurate quantization of HIV-1 RNA in each specimen. Sensitivity Current generation tests measure from 20-50 RNA copies/ml and upwards.

3.6. STATISTICAL ANALYSIS

The data obtained from this study was captured electronically in Microsoft Excel by the researcher. Any further analysis was done by an independent Biostatistician at the University of the Free State using SAS Version 9.2.1. For all the participants the following statistical analysis was done for all monthly visits.

Continuous variables were summarised by means and standard deviations or medians and percentiles (where applicable). Categorical variables were summarised by frequencies and percentages. Some continuous variables were further classified into categories (normal values, less than normal or greater than normal values) and were also described by frequencies and percentages.

The differences between the baseline screening visit and the screening at 6 months as well as the baseline screening visit and the screening at 12 months (final visit) were calculated for continuous variables and summarised by medians and percentiles. The paired median differences were compared using p-values obtained from the Wilcoxon Signed Rank test. Non-parametric confidence intervals for the paired median difference were also calculated.

The change in categorical variables, classified according to normal ranges, from screening visit to final visit were summarised by contingency tables. The Chi-square statistic (or were applicable the Fisher's Exact Test) was used to calculate the p-value to test for differences in proportions.

The difference between the gender groups during the screening- and final visit were calculated. Gender differences for continuous variables were compared using p-values obtained from Kruskal-Wallis test and 95% non-parametric confidence intervals were calculated. Gender differences for categorical variables were compared using p-values obtained from the Chi-Square statistic (or were applicable the Fisher's Exact Test).

The Spearman correlation-coefficient was calculated to investigate the relationship between some of the variables.

The dietary intake was measured and calculated as discussed in Chapter 4 and described by medians and means. For all nutrients with an AI and RDA, the percentage of participants with intakes <67 percent or ≥67 percent of AI or RDA were calculated and summarised by frequencies and percentages.
A significance level (α) of 0.05 was used throughout this study.

3.7. SAFETY MEASURES

The following safety measures were adhered to:

3.7.1. Pre- and post study evaluation

All data from the screening, monthly and final visits were documented in a study report by the clinical investigator.

3.7.2. Adverse events

The researcher carefully monitored each participant monthly by evaluating- and summarizing all the laboratory data and the physical evaluation data. Abnormal laboratory data were addressed immediately by the clinical investigator. In addition, information on adverse events was obtained from the participants by regular questioning of each participant by the clinical staff, although no leading questions were asked. When an adverse event occurred, the researcher decided whether to withdraw the participant from the study and/or initiate appropriate treatment. After withdrawal from the study, it was ensured that the participant received appropriate medical care.

In the case of adverse events, the researcher instituted general supportive measures. The researcher reported all serious adverse events to the sponsor and

Ethics Committee by telephone or facsimile within 24 hours of becoming aware of the occurrence of such an event. The notification by telephone was followed by a detailed, written report within 48 hours after the initial notification or at latest on the following working day. All serious events were followed up until the outcome was known.

The researcher ensured that the nature, date, time of onset, duration, intensity relationship to the study supplement, action taken, and outcome of all serious adverse events were documented and discussed in detail in the study report.

No adverse event regarding the supplement was reported in any of the participants.

The most common intervention applied, was the treatment of bacterial and fungial infection with appropriate medication prescribed by a medical doctor.

3.7.3. Premature discontinuation of the study

The sponsor or principal investigator had the right to discontinue the study at any time for medical and/or administrative reasons.

3.8. ETHICAL ASPECTS AND CLINICAL PRACTISE COMPLIENCE

The following ethical aspects and clinical practise compliance prevailed:

3.8.1. Mandatory approval

Written approval for the final version of the protocol was obtained from the Ethics Committee and the MCC before commencing the study (ETOVS number: ETOVS 142/05).

3.8.2. Good clinical practice (GCP)

The trial was conducted in compliance with the protocol and the following recommendations and guidelines were adhered to: South African Good Clinical

Practice Guidelines, guidelines for good clinical practice in the conduct of clinical trials with human participants in South Africa. Department of Health: Pretoria, South Africa.

3.8.2. Confidentiality

All information obtained during the conduct of the study with respect to the participants identification and state of health was regarded as confidential. An agreement for disclosure of any such information was obtained in writing and is included in the informed consent.

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CHAPTER 4

BASELINE DIETARY INTAKE OF HIV-INFECTED INDIVIDUALS LIVING IN THE MANGAUNG METROPOLITAN

ABSTRACT

The aim of this part of the present investigation is to gain information on the Baseline dietary intake of 40 HIV-infected individuals living in Mangaung Metropolitan, Free State Province, South Africa.

Dietary intake is determined using a validated Quantitative Food Frequency Questionnaire (adapted from the THUSA study, Potchefstroom University with a reliability of 90%) to determine the habitual types and quantities of food and liquid intakes for the period six months prior to subjecting the individuals to the intervention. The intake of both macronutrients and micronutrients are assessed by entering the data into a standard analytic program (FoodFinder 3, Medical Research Council).

The results of the present investigation show that the mean and/or median Energy (kJ) intake is higher than the specified Estimated Energy Requirement (EER) in both groups (males and females). The macronutrient (total protein, carbohydrate and fat) intakes in both males and females are higher than the Recommended Dietary Intake (RDA) and/or Adequate Intake (AI) specifications. The majority of the respondents consumed adequate amounts of vitamins with the exception of *Vitamin D* where 83.3% of the males and 60.6% of the females (64.1% when combined,) consumed ≤67% in terms of the RDA specification.

With the exception of *Iodine*, the dietary intake of minerals and trace element met or exceeded the specified RDA or applicable Adequate Intake (AI) specifications: Iodine intake in males is lower in terms of the mean (±SD) and median (inter-quartile range) i.e. 66.2±27.1 and 60.5 (43-87) respectively when compared to the RDA of 150µg. Iodine intake in females is lower in terms of the mean (±SD) and median (inter-quartile range) i.e. 90.2±36.7 and 88 (65-110) respectively when compared to the RDA of 150 µg. In the male group 83.3% of the individuals and 66.7% of the individuals in the female group consumed ≤67% of the RDA. When the groups are combined, 69.2% of the individuals consumed ≤67% of the RDA.

The present results suggest that an inadequate Vitamin D and Iodine intake appears to be the only limiting constraints observed in the present investigation.

4.1 LITERATURE SURVEY

Sub-Saharan Africa, housing 67% of the HIV-infected people worldwide and with Southern and East Africa where the prevalence of HIV-infected individuals probably exceeds 25% of the local population, are prone to food shortages that may contribute to malnutrition (Ahoua, Umutoni, Huerga, Minetti, Szumilin, Balkan, Olson, Nicholas & Pujades-Rodrigues, 2011). Food insecurity is defined as "Persistent lack of access to adequate food in needed quantities and quality." Food insecurity and increased nutritional vulnerability are major driving forces for nutrition care and support programs for HIV and AIDS-infected individuals (Ivers, Cullen, Freedberg, Block, Coates & Webb, 2009). The study by Ahoua and colleagues (2011) reported that 15% of HIV-infected individuals in sub-Saharan Africa require urgent nutritional rehabilitation. Nutritional therapy is therefore important in the clinical care of HIV-infected individuals and by sustaining optimal nutrition, complications and progression of HIV will be reduced (Somarriba, Neri, Schaefer & Miller, 2010). Impaired and/or reduced nutritional status may be caused by reduced intake and availability of food due to the effect of HIV and AIDS on the household income (Lemke, 2005), but also due to physiological symptoms such as oral thrush; vomiting; appetite loss; reduced nutrient absorption due to diarrhea and intestinal damage (Oketch, Paterson, Maunder & Rollins, 2011).

Healthy males and females respectively need at least 12,881 KJ and 10,093 KJ per day (Nutrient Reference values for Australia and New Zealand, 2006), of which 10%-20% should comprise protein. Unfortunately protein intake makes out a very small portion of the daily diet of many communities in Southern Africa (Spencer, Harman, Botha, Rollins, Labadarios & Visser, 2008). In order to maintain body weight and physical activity levels, a ten (10%) increase in energy requirement is

noted in asymptomatic HIV-infected individuals but is dependent on the stage of disease progression.

Vitamins and minerals are substances that are not used to provide energy, but assist the body in utilizing macronutrients for anabolic and/or catabolic purposes. In most instances vitamins act as coenzymes. With the exception of vitamin D and the small amounts of vitamin B and vitamin K that are produced by intestinal bacteria, all vitamins are obtained from food or supplementation. Minerals are needed in relatively moderate amounts and make up approximately 4% of the body weight (Marieb & Hoehan, 2010). The reduction and deficiency of vitamins A, B_6, B_{12}, C and E as well as the minerals zinc and selenium have been shown to compromise the immune response (Campa & Baum, 2010).

HIV breaks down the immune system and promotes nutritional deficiency in infected individuals (Campa & Baum, 2010). The origin of malnutrition in HIV-infected individuals is multifaceted: Acute and chronic physiological changes associated with psychological, social and economic factors play an important role in every individual's nutritional status. These complex interactions often coexist geographically (Sztam, Awzi & Duggan, 2010). The optimal nutritional status of HIV-infected individuals is important, because malnutrition is associated with an increased death rate (Leah & Mascioly, 1995; Ockenga, Grimble, Jonkers-Schuitema, Macallan, Melchior, J-C., Sauerwein & Schwenk, 2006). HIV infection has a negative influence on the human metabolism even in the early stage of the infection when apparent clinical symptoms are not visible. Chronic inflammation caused by infection increases the metabolic needs of the human body and may lead to the depletion of essential nutrients transpiring in a malnourished person with reduced immunity (Szetela & Gasiorowski, 2010).

To maintain good health a person requires foodstuffs comprised of sufficient amounts of carbohydrates, fat and protein as well as water, minerals and vitamins (Spencer *et al.,* 2008). HIV and AIDS relegate the nutritional status as being a "wastage syndrome". In accordance, sufficient amounts of micro- and macronutrients intakes in healthy individuals could become insufficient in HIV-infected and AIDS individuals at a later stage. Loss of body cell mass and muscle mass may be prevalent in the early course of HIV infection suggesting that the loss is due to the HIV infection and not opportunistic co-infections (Faintuch, Soeters & Osmo, 2006). The nutritional effects of HIV infection coincides with an increase in resting energy expenditure (during infections), decreased food intake, depression, anorexia, malabsorption, decreased quality of life, decreased work productivity and less dietary diversity, all contributing to the failure to gain weight (Sztam *et al.,* 2010). Malabsorption of fats and carbohydrates is well documented in HIV-infected individuals. This, in turn, reduces the absorption of other nutrients such as fat-soluble vitamins (A, D, E and K), endangering the immune system and aggravating the nutritional deficiency and the inflammatory reaction due to the HIV infection (Ivers *et al.,* 2009).

HIV and AIDS affect the metabolism of insulin and glucagon leading to muscle wasting (Ivers *et al.,* 2009). Blood chemistry changes are reported in HIV-infected individuals with anthropometric indices and nutrient intake within normal limits. Lower blood levels of hemoglobin, high-density lipoprotein cholesterol, total cholesterol, triacylglycerols and albumin are reported (Faintuch *et al.,* 2006). It has been reported that in HIV-infected individuals with CD4 counts below 400cells/mm^3 the blood levels of saturated fatty acids increase and the unsaturated fatty acids decrease (Faintuch *et al.,* 2006). Reduced immunity is enhanced as oxidative stress becomes more prominent. The immune changes in HIV-infected individuals, such as a decline in numbers, as well as the CD4:CD8 ratio are also found in malnourished individuals. The increasing risk of death in a malnourished HIV-infected individual is independent from the viral load and CD4 cell count (Grimble, 2009).

Nutritional assessment and supplementation with both micro- and macronutrients may support the efficacy of medical treatment in HIV-infected individuals by improving the physiological function of the body (Somarriba *et al.,* 2010). Supplements have to be administered with care as tolerable upper limits above RDA and AI exist (Monsen, 2000). All HIV-infected individuals should undergo a comprehensive nutritional assessment during their first visit to health care professionals. Proper nutritional intervention and ongoing monitoring should follow the initial assessment (Anabwani & Navario, 2005). Biochemical analysis will help to confirm the diagnoses: Reductions in the following biochemical markers are typical of malnutrition: albumin, hemoglobin, urea, creatinine, glucose, triglycerides, zinc, iron, selenium, vitamins A, B, D, folic acid, (Szetela & Gasiorowski, 2010) and vitamin E (Mehta, Spie, About, Giovannucci, Msamanga, Hertzmark, Mugusi, Hunter & Fawzi, 2010). It has been noted that selenium and vitamin A (antioxidants) are the first to decrease (Faintuch *et al.,* 2006). Signs and symptoms of malnutrition commonly include: a BMI below 18.5 kg/m^2, cell mass loss (lean body mass (Ivers *et al.,* 2009) of more than 5% within three months, lack of weight gain, hormonal abnormalities and active opportunistic infections (Ockenga *et al.,* 2006).

4.2. METHODOLOGY

The following section will provide information regarding the material and methods used.

4.2.1. Study design and demographic information.

Information based on the study design (see 3.1.1), study site (see 3.1.2), study population (see 3.2) and intervention (see 3.3) is described in Chapter 3.

4.2.2. Measuring tool and variables

The questionnaire that 40 HIV-infected individuals are subjected to is revealed in **APPENDIX D**.

4.2.3. Statistical analysis of Demographic and Absolute (measured) results

The following variables were subjected to descriptive statistical analysis and elaborated on separately and specified for both genders separately (and pooled):

- Baseline energy, macronutrient and cholesterol intake;
- Baseline vitamin (fat- and water soluble) intake; and
- Baseline mineral and trace element intake.

The data is subjected to statistical analysis and the following calculations are implemented:

- Percentage of individuals with a nutrient intake of ≤ 67% of the RDA/AI;
- The mean (±standard deviation); and
- Median (inter-quartile range).

4.3. RESULTS

This section reveals the demographic and relative results respectively.

4.3.1 Demographic results

A total of 132 HIV-infected individuals were enrolled into this investigation. One hundred and eight (108) individuals dropped out because of non-compliance to the protocol (not taking supplement correctly or missing appointments) (see Chapter 3: section 3.2.5).

Seven of the 40 participants (17%) who completed the food frequency questionnaire were male and 33 of the 40 participants (83%) were female.

4.3.2 Absolute (measured) results

The mean (±standard deviation), median (inter-quartile range), RDA/AI, and the percentage of individuals with a nutrient intake of ≤ 67% of the RDA/AL are outlined for the following variables that will be elaborated on separately and specified for both genders separately (and pooled):

- Baseline energy, macronutrient and cholesterol intake;
- Baseline vitamin (fat- and water soluble) intake; and
- Baseline mineral and trace element intake.

4.3.2.1 Baseline energy, various macronutrients and cholesterol intake

The results for Baseline energy, the various macronutrients and cholesterol are summarized and presented in **Table 4.1**. Each and every variable is discussed separately.

Baseline energy

The mean (±SD) and median (inter-quartile range) for the total energy intake for males are 20,318.7±7,602.0 and 22,583. (12,358.0 – 25,870.0 respectively). The mean (±SD) and median (inter-quartile range) for the total energy intake for females are 21,293.8±6,220.9 and 22,328.0 (17,632.0 – 26,255.0) respectively. Both genders exceeded the respective average RDA/AI

of 12,881 and 10,093.

Total protein intake

The mean (±SD) and median (inter-quartile range) for the total protein intake for males are 160.8±67.3 and 146.5 (113.9-201.5) respectively. The mean (±SD) and median (inter-quartile range) for the total protein intake for females are 180±66.6 and 174.7(140.5-225.9) respectively. Both genders exceeded the average. In both of the male and female groups the median and mean total protein intake exceeded the RDA of 46 g/day.

Table 4.1. Baseline energy, macronutrient and cholesterol intake of HIV-infected individuals in Mangaung (n=40)

VARIABLE	STATISTICAL ANALYSIS					
MACRONUTRIENTS	**Mean**	**SD**	**Median**	**Inter-quartile range**	**RDA /AI**	**% of individuals ≤ 67% of RDA / AI**
Energy (kJ)						
Male	20319	7602	22583	12,358.0 – 25,870.0	12881[a]	0
Female	21294	6221	22328	17,632.0 – 26,255.0	10093[a]	0
All	21144	6350	22328	16,867.0 – 26,255.0		
Total protein (g)						
Male	160.8	67.3	146.5	113.9 – 207.5	46[b]	0
Female	180	66.6	174.7	140.5 – 225.9	46[b]	0
All	177	66.2	172	125.8 – 225.9		0
Plant protein (g)						
Male	61.4	27.3	72.1	32.4 – 82.9	–	–
Female	54.9	21.2	53.4	39.7 – 64.4	–	–
All	55.9	22	54.4	38.3 – 74.2	–	–
Animal protein (g)						
Male	95.2	48.9	87.6	49.2 – 128.2	–	–
Female	112.2	56	108.1	69.5 – 162.1	–	–
All	109.6	54.8	107.3	60.8 – 162.1	–	–
Total fat (g)						
Male	188.4	81.7	180.3	113.1 – 262.7	–	–
Female	213.1	81.5	210.5	163.4 – 262.7	–	–
All	209.3	80.9	205.9	148.8 – 262.7	–	–
Carbohydrate (g)						
Male	572.5	223.4	588.8	352.3 – 758.9	130[b]	0
Female	559.7	171.4	581.9	479.4 – 660.1	130[b]	0
All	561.7	177	581.9	470.2 – 664.3		
Total dietary fiber (g)						
Male	48.7	21.2	60.1	30.6 – 63.4	38[c]	16.7
Female	46.7	17.7	46.8	37.4 – 59.7	25[c]	6.1
All	47	18	47.3	32.1 – 61.2		7.7
Cholesterol (mg)						
Male	420	281.5	324.5	203.0 – 668.0	< 300[d]	0
Female	822.4	470.4	781	559.0 – 1 101.0	< 300[d]	0
All	760.5	467.4	680	422.0 – 960.0		0

[a] = EER indicates Estimated Energy Requirement
[b] = RDA indicates Recommended Dietary Intake
[c] = AI indicates Adequate Intake
[d] = Recommended guideline

Fat intake

The mean (±SD) and median (inter-quartile range) for the total fat intake for males are 188.4±81.7 and 180.3 (113.1-262.7) respectively. The mean (±SD) and median (inter-quartile range) for the total fat intake for females are 213.1±81.5 and 210.5(163.4-262.7) respectively. The total fat intake of both groups (male and female) is higher than the recommended intake of less than 30% of the total daily energy intake (Gallagher, 2012).

Carbohydrate intake

The mean (±SD) and median (inter-quartile range) for the total carbohydrate intake for males are 572.5±223.4 and 588.8 (352.3-758.9) respectively. The mean (±SD) and median (inter-quartile range) for the total carbohydrate intake for females are 559.7±171.4 and 581.9(479.4-660.1) respectively. The mean and median total carbohydrate intake of both groups is more than 4 times the RDA of 130 g/day.

Fiber intake

The mean (±SD) and median (inter-quartile range) for the total fiber intake for males are 48.7±21.2 and 60.1(30.6-63.4) respectively. The mean (±SD) and median (inter-quartile range) for the total fiber intake for females are 46.7±17.7 and 46.8(37.4-59.7) respectively.

In the male group 16.7% and 6.1% of the female group consumed ≤67% of the respective recommended 38g and 25g of fiber per day.

Cholesterol

The mean (±SD) and median (inter-quartile range) for the total cholesterol intake for males are 420±281.5 and 324.5(203-668) respectively. The mean (±SD) and median (inter-quartile range) for the total cholesterol intake for females are 822.4±470.4 and 781(559-1101) respectively.

On average the total dietary intake of cholesterol in both the male and female groups is more than the recommended guideline of <300 mg/day for both genders.

4.3.2.2. Baseline vitamin (fat- and water soluble) intake

The results for the various Baseline fat- and water soluble vitamin intakes for males and females are summarized and presented in **Table 4.2.** A holistic approach is followed and only differences below or above the RDA/AL is discussed.

Table 4.2. Baseline fat- and water soluble vitamin intake of HIV-infected individuals in Mangaung (n=40)

VARIABLE	STATISTICAL ANALYSIS					
MICRONUTRIENTS	Mean	SD	Median	Inter-quartile range	RDA /AI	% of individuals ≤ 67% of RDA / AI
Vitamin C (mg)						
Male	239.7	149.4	217.5	180.0 – 250.0	90	0
Female	214.6	128.9	206	122.0 – 292.0	75	9.1
All	218.4	130.4	208	122.0 – 292.0		7.7
Thiamin (mg)						
Male	2.3	0.9	2.6	1.3 – 3.0	1.2	0
Female	2.7	1	2.6	2.2 – 3.2	1.1	3
All	2.6	1	2.6	2.1 – 3.2		2.6
Riboflavin (mg)						
Male	2.7	0.9	2.9	1.9 – 3.1	1.3	0
Female	3.8	2.2	3.4	2.4 – 4.7	1.1	6.1
All	3.6	2	3.3	2.2 – 4.7		5.1
Niacin (mg)						
Male	42.1	16.3	38	30.7 – 59.7	16	0
Female	44.8	16.2	43.3	35.2 – 53.1	14	3
All	44.4	16	42.9	32.1 – 54.7		2.6
Vitamin B6 (mg)						
Male	3.1	1.4	2.7	2.3 – 4.4	1.3	0
Female	3.4	1.3	3.4	2.9 – 4.2	1.3	3
All	3.3	1.3	3.2	2.4 – 4.4		2.6
Folate (µg)						
Male	391.8	201.3	423	204.0 – 570.0	400	33.3
Female	455.5	208.1	418	342.0 – 549.0	400	18.2
All	445.7	205.8	418	316.0 – 570.0		20.5
Vitamin B12 (µg)						
Male	6.1	3.9	5.2	3.0 – 8.1	2.4	0
Female	24.2	31.5	10.6	5.9 – 18.7	2.4	3
All	21.4	29.7	9.3	4.0 – 15.0		2.6
Vitamin A (µg)						
Male	3,051.80	1,813.90	3,489.50	1 419.0 – 4 500.0	900	16.7
Female	4,798.00	3,426.70	3,939.00	2 088.0 – 7 120.0	700	3
All	4,529.30	3,275.40	3,937.00	2 083.0 – 7 083.0		5.1
Vitamin D (µg)						
Male	7	3.7	7.1	3.3 – 9.0	15*	83.3
Female	9.8	5.9	9.1	6.2 – 11.8	15*	60.6
All	9.3	5.7	7.8	6.1 – 11.8		64.1
Vitamin E (mg)						
Male	29.2	14.4	32.6	13.9 – 42.0	15	16.7
Female	29.3	13.3	27.8	19.6 – 39.2	15	6.1
All	29.3	13.3	30.8	18.4 – 39.9		7.7
Vitamin K (µg)						
Male	732.9	539.7	849.6	131.9 – 1 213.2	120*	16.7
Female	708.2	482.7	668.6	260.5 – 1 047.1	90*	9.1
All	712	484.3	722.4	245.3 – 1140.5		10.3

*AI indicates Adequate Intake

Fat soluble vitamins

The present results show that, except for vitamin D, other fat soluble vitamin intakes on average met or exceeded the RDA/AI. Vitamin D intake in males is lower in terms of the mean (±SD) and median (inter-quartile range) i.e. 7.0±3.7 and 7.1(3.3-9) respectively when compared to the RDA of 15 µg. Vitamin D intake in females is lower in terms of the mean (±SD) and median (inter-quartile range) i.e. 9.8±5.9 and 9.1(6.2-11.8) respectively when compared to the RDA of 15 µg. In the male group 83.3% of the individuals and 60.6% of the individuals in the female group consumed ≤67% of the RDA. When the groups are combined, 64.1% of the individuals consumed ≤67% of the RDA.

Water soluble vitamins

Analysis of the present results shows that on average all water soluble vitamin intakes met or exceeded the RDA/AI.

A reduced Folate intake is observed in 33.3% of the males and 18.2% of the females (combined: 20.5%) consuming ≤67% in terms of the RDA.

4.3.2.3. Baseline mineral and trace element intake.

The results for the various Baseline mineral and trace element intakes for males and females are summarized and presented in **Table 4.3**. A holistic approach is followed and only differences below the RDA/AL are discussed.

The present results show that, except for Iodine, all mineral and trace element intakes on average met or exceeded the RDA/AI. Iodine intake in males is lower in terms of the mean (±SD) and median (inter-quartile range) i.e. 66.2±27.1 and 60.5(43-87) respectively when compared to the RDA of 150µg. Iodine intake in females is lower in terms of the mean (±SD) and median (inter-quartile range) i.e. 90.2±36.7 and 88(65-110) respectively when compared to the RDA of 150 µg. In the male group 83.3% of the individuals and 66.7% of the individuals in the female

group consumed ≤67% of the RDA. When the groups are combined, 69.2% of the individuals consumed ≤67% of the RDA.

Table 4.3. Baseline mineral and trace element intake of HIV-infected individuals in Mangaung (n=40)

VARIABLE	STATISTICAL ANALYSIS					
MICRONUTRIENTS	**Mean**	**SD**	**Median**	**Inter-quartile range**	**RDA /AI**	**% of individuals ≤ 67% of RDA / AI**
Calcium (mg)						
Male	1,277.00	502.6	1,327.50	907.0 – 1 752.0	1000*	16.7
Female	1,359.10	605.5	1,544.00	896.0 – 1 752.0	1000*	15.2
All	1,346.40	585.6	1,374.00	896.0 – 1 752.0	-	15.4
Iron (mg)						
Male	25.8	12.8	20.6	18.7 – 36.3	8	0
Female	28.7	12.1	26.4	20.5 – 39.0	18	9.1
All	28.3	12.1	24.6	19.3 – 39.0	–	7.7
Magnesium (mg)						
Male	670.5	263.3	807.5	382.0 – 862.0	420	0
Female	631.4	193.9	647	524.0 – 732.0	320	3
All	637.4	202.4	647	516.0 – 789.0		2.6
Zinc (mg)						
Male	19.2	7.4	20.3	12.1 – 22.5	11	0
Female	22.4	8.9	22.6	15.4 – 30.0	8	3
All	21.9	8.7	22.4	15.1 – 30.0		2.6
Copper (mg)						
Male	2.9	1.2	3.2	1.6 – 3.5	0.9	0
Female	3.8	2.6	3.3	2.4 – 4.0	0.9	3
All	3.7	2.5	3.3	2.3 – 4.0		2.6
Chromium (µg)						
Male	116.4	51.9	124.7	61.1 – 166.9	35*	0
Female	123.8	55.8	127.8	89.0 – 161.7	25*	3
All	122.6	54.6	125	84.2 – 165.1		2.7
Selenium (µg)						
Male	65.4	39.3	48.6	36.4 – 94.2	55	33.3
Female	94.1	56.3	84	58.0 – 125.1	55	18.2
All	89.7	54.6	80.4	46.4 – 125.1		20.5
Iodine (µg)						
Male	66.2	27.1	60.5	43.0 – 87.0	150	83.3
Female	90.2	36.7	88	65.0 – 110.0	150	66.7
All	86.5	36.2	87	64.0 – 106.0		69.2

*AI indicates Adequate Intake

4.4. DISCUSSION

The results of the present investigation reflect information based on a sub-population in the Mangaung Metropolitan.

The structure-layout for the report-back and discussion of the present findings is presented in the following format:

- Section on results pertaining to Baseline energy, macronutrient and cholesterol intake;
- Section on results pertaining to Baseline fat- and water soluble vitamin intake;
- Section on results pertaining to Baseline mineral and trace element intake; and
- Synopsis of the main findings.

In order to elaborate on additional phenomena/attributes that relate to the present findings, cross-reference between these aforementioned sections is made where deemed appropriate. The present results are also compared to the results stated by other researchers.

4.4.1 Baseline energy, macronutrient and cholesterol intake (Refer to **Table 4.1**)

The present findings show that the mean and median energy intakes for both male and female HIV-infected individuals are higher than the Estimated Energy Requirement (EER). A similar finding pertaining to higher energy intakes is reported by Oguntibeju, Van den Heever and Van Schalkwyk (2005) in a trial conducted over a period of 3 months in the same region (Mangaung Metropolitan).

The higher energy intake (kJ) of the HIV-infected individuals might be explained by means of the staple food that the individuals mainly consume i.e. high portion of maize intakes *per se* and/or coinciding in combination with a relatively small (6%)

increase above the specified 30% level of energy derived from fat. The mean and median carbohydrate intake of both male and female HIV-infected individuals exceeded the RDA of 130 g/day more than four-fold. This could possibly explain the relative contribution of carbohydrate towards high energy intake of the individuals.

Carter (2012) states that high energy intake may reduce the possibility of muscle wasting and promotes the well-being of HIV-infected individuals. This view, however, should be appreciated within the notion put forward by Ivers *et al.,* (2009) i.e. that HIV and AIDS affect the metabolism of insulin and glucagon leading to muscle wasting. A decrease in insulin could impact on anabolic growth and catabolic (energy yielding) processes. A decrease in glucagon could negatively impact on plasma blood glucose levels.

The total mean protein intake of both groups is much higher than the specified RDA of 46 g/day, with males exceeding the RDA with 114.8 g/day and females with 134 g/day. A higher intake of dietary protein than the RDA is also reported by Kim, Rimm & Gorbach (2001) and Oguntibeju (2004).

The mean and median plant protein intake is lower compared to the animal protein intake for both groups of individuals: Expressed in terms of a percentage the differences are 36% (51%) and 51% (49%) respectively. No comparable report-back in this regard is noted in the literature.

The total fat intake of 188.4 g/day for males and 213.1 g/day for the females is 6% higher (i.e. 36%) when compared to the recommended and specified <30% daily energy intake for healthy persons. However, it should be noted that specified fat requirements for HIV-infected individuals are non-existent (National Guidelines on Nutrition for people living with HIV, AIDS, TB and other Debilitating Conditions, 2007).

The median and mean fiber intake of both the male and female group exceeded the AI. Only 16.7% of the male group and 6.1% of the female group displayed a fiber intake of ≤67% of the AI.

The median and mean cholesterol intakes in all individuals are higher than the recommended guideline. In accordance none of the individuals showed a cholesterol intake of <300 mg/day.

4.4.2. Baseline fat- and water soluble vitamin intake (Refer to **Table 4.2)**

Reductions in the following biochemical markers are typical of malnutrition: albumin, hemoglobin, urea, creatinine, glucose, triglycerides, zinc, iron, selenium, vitamins A, B, D, and folic acid, (Szetela & Gasiorowski, 2010) and vitamin E (Mehta *et al.,* 2010). In accordance, the rationale for analyzing the micronutrient composition of foodstuff intakes, relates to their respective physiological functions within the body.

A brief description on some of these functions for the various micronutrients follows and should be viewed with the present results relating to each and every one of these micronutrients:

Fat soluble vitamins

Recent research shows that **vitamin D** plays an important role in modulating innate and adaptive immune function, due to the presence of vitamin D receptors in most of the immune system cells (Baeke, Takiishi, Korf, Gysemans & Mathieu, 2010). Vitamin D is involved in innate immune system regulation. The vitamin D-activating enzyme, CYP27B1, assists in linking monocytes and pathogen-sensing mechanisms (Lagishetty, Lui & Hewison, 2011). Vitamin D, a pre-hormone, acts via metabolites like $1,25(OH)_2D$ that binds to the nuclear vitamin D receptor (Gueli, Verrusio, Linguanti, Di Maio, Martinez, Marigliano & Cacciafesta, 2012) and suppresses T-helper (Th_1) CD4 cells to enhance CD4 cell production. Toll-like receptors (TLRs) of monocytes and macrophages play an important role in innate immune response (Lagishetty *et al.,* 2011). The stimulation of the TLRs in

macrophages by anti-microbial peptides results in the conversion of vitamin D to its active form. An additional role of vitamin D is to assist with the induction of cathelicidin (a protein with c-terminal cationic anti-microbial domain) to be released at the site of infection (Beard, Bearden & Striker, 2011; Verrusio, Linguanti, Di Maio, Martinez, Marigliano & Cacciafesta, 2012).

The present results show that, except for vitamin D, other fat soluble vitamin intakes on average met or exceeded the RDA/AI. Vitamin D intake in males is lower (±50%) in terms of the mean (±SD) and median (inter-quartile range) i.e. 7.0±3.7 and 7.1(3.3-9) respectively when compared to the RDA of 15 µg. Vitamin D intake in females is also lower (±35%) in terms of the mean (±SD) and median (inter-quartile range) i.e. 9.8±5.9 and 9.1(6.2-11.8) respectively when compared to the RDA of 15 µg. In the male group 83.3% of the individuals and 60.6% of the individuals in the female group consumed ≤67% of the RDA. When the groups are combined, 64.1% of the individuals consumed ≤67% of the RDA.

In terms of the numerous aforementioned functions of Vitamin D, surely, continuous exposure to inadequate Vitamin D intake could impact negatively on the health status of the individuals in many ways. It has already been noted that in people living with HIV and AIDS, lifestyle, hormonal factors, decreased physical activity, lower intake of calcium and *vitamin D* as well as lower plasma calcium levels, all contribute to the progression of the disease (Borderi, Gibellini, Vescini, De Crignis, Cimatti, Biagetti & Tampellini, 2009; Campa & Baum, 2010).

The interactions of Vitamin D and Calcium as well as the interactions between Calcium and high protein intake are discussed in section 4.4.3.

Functions of **Vitamin A** include maintenance of the epithelium; acts as an antioxidant and helps to prevent damage to cell membranes (Yang, Yuan, Tao & Wang, 2011). Vitamin A deficiency leads to increased susceptibility to lung and gastrointestinal infections, weak response to immunization and increased HIV

progression (Cassani, Villablanca, De Calisto, Wang & Mora, 2012; Yang *et al.,* 2011). The vitamin A metabolite, all-trans retinoic acid, regulates T and B cell immune responses (Cassani *et al.,* 2012).

In the male group only 16.7% showed an intake of vitamin A ≤67% of the RDA. This figure is higher than the figure of 12.5% determined by Oguntibeju (2004). The present results show that Vitamin A intake in males is higher (±300%) in terms of the mean (±SD) and median (inter-quartile range) i.e. 3,051.8±1813.9 and 3489.5(1.419-4,500) respectively when compared to the RDA/AI of 900µg. Vitamin A intake in females is even higher (700%) in terms of the mean (±SD) and median (inter-quartile range) i.e. 4,798±3426.7 and 3,939(2088-7120) respectively when compared to the RDA of 700µg. In the male group only 16.7% of the individuals and 3% of the individuals in the female group consumed ≤67% of the RDA. When the groups are combined, 5% of the individuals consumed ≤67% of the RDA.

In terms of the numerous functions of Vitamin A and according to the present results, the dietary intake of Vitamin A appears to be non-deficient and physiological function could remain intact.

The mean and median intake of **Vitamin E** and **Vitamin K** are higher than the RDA or AI. In accordance specific functions ascribed to these two vitamins should remain intact.

Vitamin E is necessary for the functioning of the immune system by increasing the cell-mediated and humeral immune response to antigens in HIV-infected individuals. This vitamin enhances macrophage phagocytic function and resistance to viral infection (Campa & Baum, 2010). The fast proliferating immune cells are easily damaged by peroxides, thus vitamin E prevents damage to the cell membranes by reducing lipid peroxidation. Supplementation with vitamin E enhances humeral as well as mediated immunity in humans (Spencer *et al.,* 2008; Molano & Meydani, 2012).

Vitamin K is produced by the normal bacterial flora that resides in the intestines of a person and from foods – mainly green leafy vegetables. This vitamin is involved in the carboxylation of glutaminic acid, necessary for blood coagulation. In HIV, vitamin K also plays a role in the regulation of inflammation (Gallagher, 2012).

Water soluble vitamins

The **B vitamins** act as coenzymes in the tricarboxylic acid cycle. Deficiencies in this group of vitamins result in lack of energy commonly present in HIV-infected individuals, even during the early asymptomatic stage (Campa & Baum, 2010).

Vitamin C is an antioxidant with detoxification properties and assists with iron absorption (Paul, 2011). In HIV, deficiency of this vitamin will lead to impaired immunity (Dong & Imai, 2012). HIV is known to increase oxidative stress markers that damage tissue and CD4 cells. It has been reported that vitamin C supplementation delayed HIV progression and the onset of AIDS (Spencer *et al.,* 2008).

Folate and **vitamin B_{12}** are necessary for DNA synthesis, with folate acting as a coenzyme (tetrahydrofolate and dihydrofolate) used in DNA and protein synthesis. Folate converts homocysteine into cystine to prevent oxidative damage to the blood vessels (Campa & Baum, 2010).

The present results show that on averages mean (±SD) and median (inter-quartile range); all water soluble vitamin intakes met or exceeded the RDA/AI.

A reduced **Folate** intake is observed in 33.3% of the males and 18.2% of the females (combined: 20.5%) consuming ≤67% Folate in terms of the RDA/AI. This did not impact on the mean or median in any significant way. However, it should be stated that Herbert, Fong & Gulle (1990) reported that 33% of HIV-infected individuals presented with low serum folate levels and a negative folate balance.

4.4.3 Baseline mineral and trace element intake (Refer to Table **4.3**)

Calcium is not just important for good bone health but could also have an effect on weight management, weight loss, and visceral fat and fat loss: Analysis of national survey data as well as small clinical studies shows an inverse relationship between calcium intakes and body fatness i.e. the higher the calcium intake, the lower the body fatness. An adequate dietary Calcium intake may help prevent excessive fat accumulation by stimulating hormonal action that targets the breakdown of stored fat (Melanson, Sharp, Schneider, Donahoo, Grunwald & Hill, 2003). Cell membrane integrity, potential regulation of heart muscle contraction and clotting of blood are influenced by this nutrient (Lee & Nieman, 2010; Marieb & Hoehan, 2010).

Inadequate calcium significantly contributes to the development of osteoporosis. Low intakes of calcium and inadequate vitamin D status often cluster with higher prevalence rates of obesity (Soares, Murhadi, Kurpad, Piers. 2012).

In people living with HIV and AIDS, lifestyle, hormonal factors, decreased physical activity, lower intake of Calcium and Vitamin D as well as lower plasma calcium levels all contribute to the progression of the disease (Borderi, *et al.,* 2009; Campa & Baum, 2010).

On the contrary, and at first sight, the present findings show that the mean and median intake for Calcium is relatively higher than the specified RDA of 1,000 mg/day for both genders, does not exceed the and the upper limit is 2500mg per day (Curtiss & Johnson, 2007) and in effect, accordingly, raises no concern. However, a link between vitamin D and calcium absorption from the alimentary tract exists: Calcium is better absorbed when taken in combination with Vitamin D. Lack of the simultaneous intake of Calcium and Vitamin D intake in the diet impedes on the formation of the hormone calcitriol (known as the “active vitamin D”). This in turn leads to insufficient calcium absorption from the diet. In this situation, the body must take calcium from its stores in the skeleton, which weakens existing bone and prevents the formation of strong, new bone (Anderson, 2008). Although the present

results show sufficient intake of Calcium, a decrease in Vitamin D intake is observed and, accordingly, could impact on the health status of the individuals with low vitamin intake.

Furthermore, it should also be stated that although a balanced diet aids calcium absorption, high levels of protein and sodium (salt) in the diet are thought to increase calcium excretion through the kidneys. Excessive amounts of these substances should be avoided, especially in those with low calcium intake (Rolfes, Whitney, & Pinna, 2009).

The present results indicate a relatively high protein intake: The mean (±SD) and median (inter-quartile range) for the total protein intake for males are 160.8±67.3 and 146.5 (113.9-201.5) respectively. The mean (±SD) and median (inter-quartile range) for the total protein intake for females are 180±66.6 and 174.7(140.5-225.9) respectively. Both genders exceeded the average in both male and female groups the median and mean total protein intake exceeded the RDA of 46 g/day.

In accordance, interactions between micronutrients and macronutrients exist and should not be viewed as single entities *per se:* Calcium intake appears to be sufficient but low Vitamin D intake could impact on Calcium metabolism in various and numerous physiological ways.

The baseline intakes for Iron, **magnesium, zinc, copper and chromium** are higher than the RDA and AI respectively. In accordance, the following physiological functions could probably remain intact: Magnesium, zinc and copper are cofactors and components of more than 300 enzyme systems. Magnesium is widely present in green vegetables; zinc in food like meat, fish and poultry. Copper is available from organ meat, seafood, nuts and seeds. Magnesium deficiency may lead to neuromuscular defects, anorexia and weight loss. Zinc depletion results in endogenous zinc loss resulting in diarrhea, reduced appetite and impaired immune response. Copper deficiency results in hyperchromic anemia, leucopenia,

neutropenia and may negatively affect the cardiovascular and immune systems. Chromium is associated with glucose metabolism and a deficiency enhances diabetes and cardiovascular disease (Nutrient Reference values for Australia and New Zealand, 2006).

The intake of **selenium** is inadequate in 33.3% of males and 18.2% of females i.e. values less than ≤67% of the specified RDA standard measured. In accordance, the following physiological functions could be strained:
Selenium is an important component of the humeral immune repose through its active role in interleukin-2 and cytokine action in the expression of T-Lymphocyte expansion. Furthermore, selenium inhibits the progression and reduces the viral load of HIV by means of its antioxidant effect through the enzymes glutathione peroxidase and thioredoxin reductase where selenium functions as an active component in the processes involved. These enzymes reduce oxidized molecules, thereby protecting cells from oxidative stress caused by reactive oxygen radicals Campa & Baum (2010). Reduced selenium status is associated with an increase of HIV disease progression and mortality (Pitney, Royal & Klebert, 2009; Irlam, Visser, Rollins & Siegfried, 2010; Stone, Kawai & Kupka, 2010). Selenium is a chemo preventive mediator in HIV that may increase the defense systems of the human body, reduce viral load (Campa & Baum, 2010) and specifically improves CD4 cell counts of these individuals (Stone *et al.,* 2010).

Iodine is a trace element and micronutrient required in the synthesis of thyroid hormones, triiodothyronine (T3) and thyroxine (T4). Thyroid hormones play a major role in the metabolic processes of the body (Leung, Braveman & Pearce. 2012). It is involved in the regulation of macronutrient metabolism and basal metabolic rate (Bougma, Aboud, Harding & Marquis, 2013). Iodine deficiency may lead to low levels of thyroid hormones resulting in full-blown hypothyroid syndrome (myxedema). Symptoms include mental sluggishness, edema and low metabolic rate (Marieb & Hoehan, 2013).

The high concentration of iodine in the thymus supports the role of this element in the immune system. Leukocyte myeloperoxidase enzymes in cell-mediated immunity use iodine to produce iodine-free radicals. Iodine also increases immunoglobulin-G production in human lymphocytes. The non-endocrine effects of iodine include increase in the movement of granulocytes into areas of inflammation and improve phagocytosis ability of the granulocytes. The immune deficiency in iodine-deficient individuals may be restored by oral administration of iodine (Venturi, 2009). According to Beltran, Lescure, El Esper, Schmit and Desailloud (2006), **iodine** deficiency in HIV-infected individuals may contribute towards hypothyroidism.

Iodine intake in males is lower in terms of the mean (±SD) and median (inter-quartile range) i.e. 66.2±27.1 and 60.5(43-87) respectively when compared to the RDA of 150µg. Iodine intake in females is lower in terms of the mean (±SD) and median (inter-quartile range) i.e. 90.2±36.7 and 88(65-110) respectively when compared to the RDA of 150 µg. In the male group 83.3% of the individuals and 66.7% of the individuals in the female group consumed ≤67% of the RDA. When the groups are combined, 69.2% of the individuals consumed ≤67% of the specified RDA standard.

4.4.4 Reflection of the findings in terms of the composition of the supplement.

The researcher had no indication or any insight knowledge on the baseline dietary intake levels of the 40 HIV-infected individuals prior to the formulation of the product. Furthermore, the researcher did not participate in formulating the product and viewed the formulation as being an arbitrary concoction of ‘nutritional substances’ where claims are made in terms of the ‘beneficial effects’ ascribed to use of the supplement by HIV-infected individuals. The primary goal is to evaluate if the supplement impact on various and numerous variables indicative of the health status of HIV-infected individuals (see chapter 5).

The ingredients and their respective quantities that comprise the product are revealed in Chapter 3: 3.3.2.

In retrospect/hindsight, and at this point in time, it appears to be easy to predict what the possible outcome in terms of the presence of the micronutrient components of formulation should entail i.e. the present results show a decrease in the intake of Vitamin D and Iodine and should have been present (not absent) in the formulation. All other micronutrients on average (mean and median) met or exceeded the RDA/AI and one could consider supplementation as being an unnecessary additive/venture. In terms of the ingredients other than micronutrients, i.e. Biociden, Colostrum, and Spirulina lend elaboration will merely lend itself to speculation only. The nutritional requirements of HIV-infected individuals are most probably higher than the recommendation for healthy persons.

4.5 CONCLUSION

The main findings of the present investigation suggests that the eating pattern of this urbanized group of individuals reflects a high dietary intake of proteins and carbohydrate coinciding with a decrease in Vitamin D and Iodine intake in a significant number of individuals.

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CHAPTER 5

INFLUENCE OF NUTRITIONAL SUPPLEMENT INTAKE ON THE HAEMATOLOGICAL STATUS, IMMUNE STATUS AND VIRAL LOAD IN HIV-INFECTED INDIVIDUALS LIVING IN THE MANGAUNG METROPOLITAN

ABSTRACT

The aim of the investigation was to determine if supplementation with a mixture comprised from specific minerals, vitamins and herbs over a period of one year, affect the viral load, immune and Haematological status in forty (40) HIV-infected individuals living in the Mangaung Metropolitan.

The study commenced with a screening visit for inclusion purposes, followed by a baseline evaluation on numerous variables. Participants received the supplement with their monthly clinic visits for a period of 12 months. Blood samples were obtained, clinical assessment and pulmonary function tests performed during all monthly visits.

The results show statistical significant median difference ($p<0.0001$) decreases in the mean viral load values of the study population i.e. from 51,040 RNA copies per milliliter plasma to 43,107 RNA copies per milliliter plasma over the first 6-month period and 37,307 RNA copies per milliliter plasma over the 12 month period respectively.

From all of the haematological variables the only statistical significant changes observed are increases in the median ESR ($p=0.0219$) and MCHC ($p=0.0245$) and a decrease in viral load ($p=0.0001$) after 6 months. At 12 months a statistical significant decrease in the median CD/CD8 ratio ($p<0.0048$), median Hematocrit concentration ($p<0.0312$), median MCV ($p<0.0359$), and median RDW ($p<0.0273$) accompanies the significant decrease in the median viral load ($p<0.0001$). The only variable(s) that show a statistical significant increase is the MCHC ($p<0.0003$) at 12 months.

The study population was divided into two (2) sub-populations demarcated to present positive responders (i.e. decrease in viral load) and non-responders (no change or increase in viral load). At 6 months 89% ($CI_{95\%}$: 73%;96%) of the individuals show a decrease in viral load counts with a median percentage change

of 34% ($CI_{95\%}$: 73%;96%). At 12 months 85% [$CI_{95\%:}$ 68% ; 94%] of the individuals show a decrease in viral load counts with a median percentage change of 62.9% ($CI_{95\%}$: 50%;78.6%) following the intake of the supplement. In terms of the decrease in viral load and the number of individuals showing a decrease in viral load following use of the supplement, the present results suggest that those measurable clinical advantages are not related to chance/coincidence.

5.1 INTRODUCTION

Nutritional metabolism and human immunity are complex biological systems that have to function in a synergistic fashion to sustain and preserve life. Any disruption of the nutritional status of any individual will be detrimental to the ability of the immune system to protect the human body against infection. A reduction in immune system activity will also impact negatively on the nutritional status of the immune compromised individual (Afacan, Fjell & Hancock, 2012). The immune system requires essential nutrients to produce needed defense mechanisms and defense cells (Carter, 2011). An inadequate intake of specific micronutrients, suppresses the immune system by reducing the innate T-cell response leading to deregulation of the host immune response (Wintergerst, Maggini & Hornig, 2007).

HIV is known to break down the immune system and promotes nutritional deficiency in infected individuals (Campa & Baum, 2010). HIV changes the production of T-cell precursors that impair CD4 cell response resulting in opportunistic infections and AIDS (De Biasi, Pinti, Nasi, Gibellini, Bertoncelli, Manzini, Mussini & Cossarizza, 2011). The weeks that follow the initial infection are associated with prompt damage to the immune cells due to direct viral cell toxicity (McMichael, Borrow, Tomaras, Goonetilleke & Haynes, 2010).

The first stage of HIV infection is the attachment of HIV glycoprotein envelope to the primary cellular receptor, CD4 cells. This binding leads to viral and host membrane fusion with viral capsid entrance into the host cells (Friedrich, Dziuba, Li, Endsley, Murray & Ferguson, 2011). HIV enters the host cell also by means of endocytosis

by binding to the lectins on the cell surface. After the viral core is released into the cytoplasm of the host cell, reverse transcriptase converts the virus RNA to DNA (Yan & Lieberman, 2011).

The human innate immune response is first activated by HIV and then the adaptive immune response is triggered. The innate immunity is activated by the recognition of pathogen-associated molecular patterns (Yan & Lieberman, 2011).
The CD8 cells are vital to control viral replication by secreting interferon gamma that induces the production of antiviral proteins and immune response that destroys infected cells. This implies that a strong cytotoxic T-Lymphocyte reaction is associated with improved virus control and delayed disease development during early HIV-infection (Kim, Lee, Hong & Kim, 2010).

HIV escape from the CD8 cell response may occur very quickly, even within 10 days of lymphocyte recognition. This commences with viral cell reservoirs and antibody escaped viral mutants emerge in the host plasma that may lead to the activation of uninfected CD4 cells and death by apoptosis, causing immune suppression. Up to 80% of the host CD4 cells can be depleted in the first three weeks of HIV infection (McMichael *et al.,* 2010). The majority of untreated HIV-infected individuals develop uncontrolled viremia with progressive immune destruction and ultimately AIDS and death (Théze, Chakrabarti, Vingert, Porichis & Kaufmann, 2011).

Significant weight loss and underweight are widely present among HIV-infected adults in sub-Saharan Africa. This compromised nutritional status may result from reduced food intake due to the impact of HIV on the health of the infected individual. The weight loss in HIV-infected individuals is potentiated by elevated tumor necrosis factor alpha, interleukin-1 and interleukin-6 (Koethe, Chi, Megazzini, Heimburger & Stringer, 2009).

The compromised immune function leads to opportunistic infections such as oral thrush and diarrhoea leading to reduced nutritional intake. Other factors include fatigue, nausea, vomiting, appetite loss, skin infection and prolonged fever. Food insecurity also contributes towards malnourishment of HIV-infected individuals (Oketch, Paterson, Maunder & Rollins, 2011).

Malnutrition has a negative effect on the innate, adaptive as well as cellular immune response leading to reduced host defense and increased pathogen virulence. In turn, the infection negatively impacts on the micronutrient status of the body by reducing nutrient intake, interfering with metabolic pathways and excessive loss of nutrients (Wintergerst *et al.,* 2007). A study by Afacan and associates (2012) suggests that viruses modify the metabolic processes of infected cells by increasing aerobic glycolysis, resulting in a shift of the cellular metabolism to enhance energy promotion over cell biosynthesis.

A study regarding the nutritional status of HIV-infected individuals show that malnutrition contributing to weight loss in HIV-infected individuals plays a predictive roll in disease progression regardless indicators such as low CD4 cell count (Venter, Gericke & Bekker, 2009). Malnutrition in impaired immunity interrupts recovery from disease and increases complications associated with the infection (Cawood, Elia & Stratton, 2012).

Research conducted in the Mangaung Metropolitan show that micronutrient deficiencies occurred in the majority of HIV-infected children in care centers (Steenkamp, Dannhauser, Walsh, Joubert & Veldman, (2009). The prominent deficient micronutrients are zinc, Vitamin A, Vitamin D and glutathione and the researchers recommend vigorous macro- and micronutrient supplementation in an attempt to eliminate these nutrient deficiencies. The first line of defense against pathogenic invasion is the innate immune system. Evidence by Lagishetty, Lui and Hewison (2011) show that Vitamin D is involved in innate immune system

regulation. Vitamin D-activating enzyme (CYP27B1) assists in linking monocytes and pathogen sensing mechanisms (Lagishetty, Lui & Hewison, 2011).

The importance of nutrient supplementation is highlighted by many HIV and AIDS programmes in sub-Saharan Africa that started to include nutritional assistance in addition to medical treatment to reduce malnutrition and improve the outcome in HIV-infected individuals (Tirivayi & Groot, 2011). The improvement of the nutritional status of HIV-infected individuals improves their physiological and psychological status with better quality of life (Oketch *et al.,* 2011).

The aim of the investigation was to determine if supplementation with a mixture comprised from specific minerals, vitamins and herbs over a period of one year, affect the haematological status, immune status and/or viral load in forty (40) HIV-infected individuals living in the Mangaung Metropolitan.

5.2. METHODOLOGY

The following section will provide information regarding the material and methods used.

5.2.1. Protocol design demographic information.

Information based on the study design (see 3.1.1), study site (see 3.1.2), study population (see 3.2) and intervention (see 3.3) is described in Chapter 3.

5.2.2. Measuring tool, variables and analysis of data

Information pertaining to the apparatus used and the various haematological and immunological variables including the viral load are made by means of using standard accredited laboratory and technological procedures and is disclosed in Chapter 3 section 3.5.2.

5.2.3. Statistical analysis of data

The data is subjected to statistical analysis categorized into two populations:

1. A study population where the following statistical formulations are applied:
    - Percentages and corresponding 95% confidence intervals for these percentages (descriptive statistics);
    - Mean and corresponding standard deviations;
    - Median and 95% confidence intervals for the mean; and
    - Corresponding p-values for median differences and 95% confidence intervals for these mean differences.

2. A study subpopulation category demarcated as responders and non-responders in terms of changes in viral load is drafted where the following statistical formulations are applied:
    - Percentages and corresponding 95% confidence intervals for the percentages;
    - Median percentage change and 95% confidence intervals for the .median percentage change and
    - Corresponding p-values for median differences and 95% confidence intervals for these mean differences.

Baseline levels are obtained for all variables and compared to the various corresponding levels at 6 months and 12 months respectively.

5.3. RESULTS

The following sections reflects the demographic, absolute and relative results.

5.3.1. Demographic results

A total of 132 HIV-infected individuals were enrolled into present investigation but due to dropouts the study is completed with a final total of 40 individuals. The relatively high dropout figure can be ascribed to noncompliance with the protocol design i.e. not taking the supplement correctly or missing appointments–see Chapter 3 section 3.5.2. Seven (17%) of the 40 participants are male while 33 (83%) are female.

5.3.2. Relative results

Relative results refer to calculated values derived from specific measurements. The results obtained for two (2) populations are revealed i.e. entire study population and the two sub-populations.

5.3.2.1. Results for the Study Population category (SP)

Table 5.1 indicates the normal values, mean, standard deviation, median and p-values (statistical significant differences highlighted in red) for viral load, haematological, and immunological variables at baseline, six (6) months, and twelve months (12) for the entire population.

The results show statistical significant median difference ($p<0.0001$) decreases in the mean viral load values of the study population i.e. from 51,040 RNA copies per milliliter plasma to 43,107 RNA copies per milliliter plasma over the first 6-month period and 37,307 RNA copies per milliliter plasma over the 12 month period respectively. From all of the haematological variables the only statistical significant changes observed are increases in the median ESR ($p=0.0219$) and MCHC ($p=0.0245$) and a decrease (in Viral load ($p=0.0001$) after 6 months. At 12 months a statistical significant decrease in the median CD/CD8 ratio ($p<0.0048$), median Hematocrit concentration ($p<0.0312$), median MCV ($p<0.0359$), and median RDW ($p<0.0273$) accompanies the significant decrease in the median viral load

(p<0.0001). The only variable(s) that show a statistical significant increase is the MCHC (p<0.0003) at 12 months.

Table 5.1. Haematological and immunological variables and viral load of the HIV-infected individuals at baseline, 6 months, and final visit at 12 months (n=40).

		BASELINE			6 MONTHS				12 MONTHS			
VARIABLE	Normal values	Mean	Std Dev	Median	Mean	Std Dev	Median	p-Values for median differences* (0-6 months)	Mean	Std Dev	Median	p-Values for median differences * (0-12 months)
RBC (10^{12}/l)	3.7-5.3	4.53	0.44	4.48	4.48	0.51	4.54	0.28	4.48	0.36	4.47	0.3007
Hemoglobin (g/dl)	12.0-16.0	12.96	1.45	12.95	12.89	1.4	12.7	0.3991	12.69	1.98	12.7	0.5432
Hematocrit (l/l)	0.35-0.45	0.4	0.04	0.41	0.39	0.04	0.4	0.0794	0.39	0.03	0.38	0.0312
MCV (fl)	81-100	88.11	4.92	88.5	87.29	4.27	88	0.0764	87.16	5.34	87.5	0.0359
MCH (pg)	28-35	28.5	2.06	28	28.71	2.12	29	0.1333	28.89	2	29	0.0843
MCHC (g/dl)	32-37	32.5	1.18	33	32.87	1.3	33	0.0245	33.26	1.29	33	0.0003
RDW	10--15	14.46	1.38	14.25	14.37	1.8	13.95	0.1854	14.14	1.41	13.95	0.0273
WCC (10^9/l)	4.0-11.0	5.2	2.02	4.65	5.19	1.54	5	0.9546	5.16	1.64	4.7	0.9533
Neutrophil (10^9/l)	2.0-7.5	2.33	1.32	1.84	2.45	0.99	2.17	0.2414	2.45	1.27	2.08	0.1313
Lymphocyte (10^9/l)	1.0-4.0	2.11	0.97	1.83	2	0.95	1.83	0.225	1.95	0.98	1.82	0.0563
Monocyte (10^9/l)	0.0-0.95	0.56	0.34	0.49	0.47	0.19	0.45	0.1039	0.45	0.17	0.45	0.1588
Eosinophil (10^9/l)	0.0-0.4	0.19	0.24	0.12	0.2	0.29	0.12	0.9425	0.18	0.21	0.1	0.1499
Basophil (10^9/l)	0.0-0.10	0.03	0.02	0.02	0.02	0.02	0.02	0.219	0.02	0.01	0.02	0.071
Platelets (10^9/l)	140-420	277.22	87.73	260	256.55	63.19	242	0.1544	251.37	51.75	234	0.0683
Plasma Viscosity (ESR) (mm/h)	3--12	53.7	39.99	38	47.66	31.75	39.5	0.0219	44.45	30.76	37.5	0.0921
Total T cell Count ($cm^{2)}$	940-2380	1670	711.63	1477	1667.9	856.59	1524	0.6694	1576.8	814.37	1413.5	0.5353
CD4 count ($cm^{2)}$	510-1310	386.61	137	379	395.32	151.81	361	0.9717	363.37	147.16	318.5	0.1623
CD8 count ($cm^{2)}$		1223.4	654.29	1130.5	1252.6	772.23	1036	0.5786	1199.9	731.13	1033	0.7282
CD4/CD8 (ratio)	0.85-3.13	0.37	0.16	0.32	0.36	0.15	0.34	0.0563	0.34	0.16	0.31	0.0048
Viral load (copies/ml)		51040	119609	51041	43107	45419	23790	0.0001	37307	50055	18197	0.0001

*Signed Rank Test for median differences for dependent data.
RBC Red cell count
MCV Mean corpuscular volume
MCH Mean corpuscular Hemoglobin
MCHC Mean corpuscular Hemoglobin concentration
RDW Red cell distribution width
WCC White cell count
Std Dev Standard deviation
ESR Erythrocyte sedimentation rate
p<0.05 Statistical significant difference

Seven of the most prominent variables regarded as being important in the progression of HIV-infected individuals, are selected from **Table 5.1** and is presented in **Table 5.2** (see the specified variables in the column specified as 'VARIABLES').

The statistical analysis performed emphasise the number of individuals that show a change [expressed as a percentage (%:$CI_{95\%}$)] and the 'size of change' [expressed as the median percentage change (%:$CI_{95\%}$)] indicating the type of change (increase; decrease; no change) when baseline values are compared to the corresponding and respective values obtained for the specified variables at six (6) and twelve (12) months. Relatively large changes are highlighted in red.

Table 5.2. The percentage of HIV-infected individuals with change in haematological and immunological values and viral load, at baseline, six months, and final visit at 12 months (n=40).

		0 - 6 MONTHS		0 - 12 MONTHS	
VARIABLES	CHANGE	0 to 6 months (%) $CI_{95\%}$	Median change (%) 0 to 6 months $CI_{95\%}$	0 to 12 months (%)$CI_{95\%}$	Median change (%) 0 to 12 months $CI_{95\%}$
Hemoglobin	Increase	40% [24% ; 57%]	4.1% [2.9% ; 11.2%]	42% [27% ; 59%]	4.3% [1.5% ; 12.4%]
	Decrease	55% [38% ; 71%]	4.5% [2.3% ; 6.2%]	55% [38% ; 71%]	4.5% [1.4% ; 8.8%]
	No change	5% [1% ; 19%]	-	3% [0% ; 15%]	-
Hematocrit	Increase	29% [16% ; 46%]	5.6% [2.6% ; 14.3%]	26% [14% ; 43%]	7.1% [2.6% ;12.5%]
	Decrease	55% [38% ; 71%]	5.6% [4.5% ; 9.5%]	63% [46% ; 78%]	5.1% [4.5% ; 8.2%]
	No change	16% [7% ; 32%]	-	11% [3% ; 26%]	-
WCC	Increase	53% [36% ; 69%]	20.1% [5.7% ; 38.4%]	45% [29% ; 62%]	14.6% [10.9% ; 27.8%]
	Decrease	47% [31% ; 64%]	13.4% [12.0% ; 31.4%]	45% [29% ; 62%]	14.3% [5.8% ; 23.4%]
	No change	-	-	10% [3% ; 26%]	-
CD4 count	Increase	53% [31% ; 64%]	21.8% [5.7% ; 38.4%]	34% [20% ; 51%]	21.6% [8.2% ; 50.5%]
	Decrease	47% [36% ; 69%]	18.5% [12.0% ; 31.4%]	66% [48% ; 80%]	18.2% [9.7% ; 21.1%]
	No change	-	-	-	-
CD8 count	Increase	57% [40% ; 73%]	14.8% [7.5% ; 34.9%]	59% [42% ; 75%]	10.9% [3.5% ; 27.7%]
	Decrease	43% [27% ; 60%]	22.8% [9.8% ; 31.2%]	41% [24% ; 58%]	26.7% [7.0% ; 38.1%]
	No change	-	-	-	-
CD4/CD8 ratio	Increase	29% [16% ; 46%]	14.3% [8.3% ; 35.7%]	26% [14% ; 43%]	12.9% [3.4% ; 26.7%]
	Decrease	61% [43% ; 76%]	14.0% [9.5% ; 17.9%]	66% [49% ; 80%]	14.3% [11.1% ; 20.7%]
	No change	10% [3% ; 26%]		8% [2% ; 22%]	
Viral load	Increase	11% [4% ; 27%]	62%(n is too small)	15% [6% ; 32%]	136% (n is too small)
	Decrease	89% [73% ; 96%]	34% [21.3% ; 55.2%]	85% [68% ; 94%]	62.9% [50.0% ; 78.6%]
	No change		-	-	-

Each and every variable is discussed separately:

Hemoglobin

At six months, 40%($CI_{95\%:}$ 24% ; 57%) of the individuals show a median 4.1% ($CI_{95\%:}$ 2.9% ; 11.2%) increase in hemoglobin concentration, while 55% ($CI_{95\%}$ [38% ; 71%]) show a decrease of 4.5% ($CI_{95\%}$ [1.4% ; 8.8%]). Five per cent of the individuals show no change in hemoglobin concentration.

At 12 months 42% ($CI_{95\%}$ [27% ; 59%]) of the individuals show a 4.3% ($CI_{95\%}$ [1.5% ; 12.4%]) increase in hemoglobin concentration, while 55% ($CI_{95\%}$[38% ; 71%]) show a decrease of 4.5%($CI_{95\%}$[1.4% ; 8.8%]). Three per cent of the individuals show no change in hemoglobin concentration

Hematocrit

At six months, 29% ($CI_{95\%}$[16% ; 46%]) of the individuals show a 5.6%($CI_{95\%}$ [2.6% ; 14.3%]) increase in hematocrit, while 55% ($CI_{95\%}$[38% ; 71%]) show a decrease of 5.6%($CI_{95\%}$[4.5% ; 9.5%]). Sixteen per cent of the individuals show no change.

At 12 months 26% ($CI_{95\%}$[14% ; 43%]) of the individuals show a 7.1% ($CI_{95\%}$[2.6% ;12.5%]) increase in hematocrit, while 63% ($CI_{95\%}$[46% ; 78%]) show a decrease of 5.1% ($CI_{95\%}$[4.5% ; 8.2%]). Eleven per cent of the individuals show no change.

White cell count (WCC)

At six months, 53% ($CI_{95\%}$[31% ; 64%]) of the individuals show a 20.1% ($CI_{95\%}$ [5.7% ; 38.4%]) increase in WCC, while 47%($CI_{95\%}$ [31% ; 64%]) show a decrease of 13.4% ($CI_{95\%}$[12.0% ; 31.4%]). No individuals show unchanged WCC.

At 12 months 45% ($CI_{95\%}$ [29% ; 62%]) of the individuals show a 14.6% ($CI_{95\%}$ [10.9% ; 27.8%]) increase in WCC, while 45% ($CI_{95\%}$[29% ; 62%]) show a decrease of 14.3%($CI_{95\%}$ [5.8% ; 23.4%]). Ten per cent of the individuals show no change in WCC.

CD4 count

At six months, 53% ($CI_{95\%}$[31% ; 64%]) of the individuals show a 21.8% ($CI_{95\%}$ [5.7% ; 38.4%]) increase in CD4 count, while 47% ($CI_{95\%}$[36% ; 69%]) show a decrease of 18.5%($CI_{95\%}$). No individual show an unchanged CD4 count.

At 12 months 34%($CI_{95\%}$) of the individuals show a 21.6% ($CI_{95\%}$) increase in CD4 count, while 66%($CI_{95\%}$ [48% ; 80%]) show a decrease of 18.2% ($CI_{95\%}$ [9.7% ; 21.1%]) No individual show an unchanged CD4 count.

CD8 count

At six months, 57% ($CI_{95\%}$[40% ; 73%]) of the individuals show a 14.8% ($CI_{95\%}$[7.5% ; 34.9%]) increase in CD8 count, while 43% ($CI_{95\%}$[27% ; 60%]) show a decrease of 22.8%($CI_{95\%}$[9.8% ; 31.2%]). No individual show an unchanged CD8 count.

At 12 months 59%($CI_{95\%}$ [42% ; 75%]) of the individuals show a 10.9% ($CI_{95\%}$[3.5% ; 27.7%]) increase in CD8 count, while 41% ($CI_{95\%}$[24% ; 58%]) show a decrease of 26.7% ($CI_{95\%}$[7.0% ; 38.1%]). No individual show an unchanged CD8 count.

CD4/CD8 ratio

At six months, 29% ($CI_{95\%}$[16% ; 46%]) of the individuals show a 14.3%($CI_{95\%}$[8.3%; 35.7%]) increase inCD4/CD8 ratio, while 61%($CI_{95\%}$[43% ; 76%]) show a decrease of 14%($CI_{95\%}$[9.5% ; 17.9%]). Ten (10) per cent of the individuals show no change in the CD4/CD8 ratio.

At 12 months 26%($CI_{95\%}$ [14% ; 43%]) of the individuals show a 12.9%($CI_{95\%}$ [3.4%; 26.7%]) increase inCD4/CD8 ratio, while 66% ($CI_{95\%}$[49% ; 80%]) show a decrease of 14.3% ($CI_{95\%}$[11.1% ; 20.7%]). Eight (8) per cent of the individuals show no change in CD4/CD8 ratio.

Viral load

At six months, 11% ($CI_{95\%}$[4% ; 27%]) of the individuals show a 62%(n is too small) increase in viral load, while 89% ($CI_{95\%}$[73% ; 96%]) show a decrease of 34% ($CI_{95\%}$ [21.3% ; 55.2%]). Five (5) per cent of the individuals show no change in viral load.

At 12 months 15%($CI_{95\%}$[6% ; 32%]) of the individuals show a 136% (n is to small) increase in viral load, while 85% ($CI_{95\%}$ [68% ; 94%]) show a decrease of 62.9% ($CI_{95\%}$[50.0% ; 78.6%]).

5.3.2.2. Results for the Sub-Population category (SPG)

The entire study population is sub-divided into two (2) sub-groups demarcated to present positive responders (i.e. decrease in viral load) and non-responders (no change or increase in viral load).

Table 5.3 indicate the actual measured raw data values, median difference and the respective corresponding p-values for the median difference for the specified haematological and immunological variables when baseline values are compared to the values obtained at 6 and 12 months for the non-responders **(A)** and responders (**B**) separately.

Non-responders (n=4)

No statistical significant changes in haematological or immunological values of HIV-infected individuals with a viral load increase, at baseline, six months, and final visit at 12 months are indicated (**Table 5.3 A**).

Table 5.3 Median haematological and immunological values at baseline, six months, and 12 months in non-responders (n=4) and responders (n=36) after exposure to the intervention.

A: NON- RESPONDERS (n=4)

VARIABLE	Baseline	6 months	12 months	Median Difference between Baseline and 6 months	Median Difference between Baseline and 12 months	P Value for median difference between Baseline and 6 months	P Value for median differences between Baseline and 12 months
RBC (10^{12}/l)	4.93	4.56	4.5	0.33	0.32	0.125	0.125
Hemoglobin (g/dl)	14.15	13	12.95	1.1	0.95	0.125	0.125
Hematocrit (l/l)	0.43	0.39	0.38	0.03	0.04	1	0.125
MCV (fl)	89.5	89	89	0.5	2	0.5	0.375
MCH (pg)	29.5	30.5	30.5	-0.5	-0.5	0.5	1
MCHC (g/dl)	33.5	33.5	34	0	-0.5	1	0.5
RDW	13.05	13.7	13.85	-0.15	-0.45	0.5	0.5
WCC (10^9/l)	4.2	4.45	4.3	0.3	0.1	0.625	1
Neutrophil (10^9/l)	1.92	1.92	2.04	-0.1	-0.41	0.25	0.875
Lymphocyte (10^9/l)	1.53	1.38	1.37	0.01	-0.03	1	1
Monocyte (10^9/l)	0.42	0.4	0.46	-0.01	0	0.875	1
Eosinophil (10^9/l)	0.07	0.13	0.06	0.01	0.08	0.25	0.875
Basophil (10^9/l)	0.06	0.03	0.03	0	-0.01	0.5	1
Platelets (10^9/l)	233	224	215	2	7	0.875	0.875
Plasma Viscosity (ESR) (mm/h)	18.5	26	28.5	-13	-5.5	0.875	0.125
Total T cell Count ($cm^{2)}$	1455	1283.5	1151.5	118	155.5	0.125	0.625
CD4 count ($cm^{2)}$	346.5	312.5	297.5	38	45.5	0.875	0.375
CD8 count ($cm^{2)}$	1060	869.5	785.5	114.5	84	0.875	0.625
CD4/CD8 (ratio)	0.38	0.4	0.33	0.07	0.04	0.875	0.375

B: RESPONDERS (n=36)

VARIABLE	Baseline	6 months	12 months	Median Difference between Baseline and 6 months	Median Difference between Baseline and 12 months	P Value for median difference between Baseline and 6 months	P Value for median differences between Baseline and 12 months
RBC (10^{12}/l)	4.47	4.57	4.48	0.08	0.05	0.6541	0.4363
Hemoglobin (g/dl)	12.7	12.8	12.7	0.2	0.1	0.8252	0.7592
Hematocrit (l/l)	0.41	0.4	0.39	0.01	0.01	0.2452	0.0316
MCV (fl)	89	88	87	0	1	0.1105	0.0653
MCH (pg)	28	29	29	0	0	0.3287	0.1304
MCHC (g/dl)	32.5	33	33	0	-1	0.0384	0.0013
RDW	14.35	13.9	13.9	0.45	0.55	0.0562	0.002
WCC (10^9/l)	4.7	5.05	4.7	-0.2	0	0.7215	0.8579
Neutrophil (10^9/l)	1.78	2.25	2.22	-0.26	-0.2	0.0827	0.1233
Lymphocyte (10^9/l)	1.87	1.83	1.91	0.11	0.17	0.2372	0.127
Monocyte (10^9/l)	0.49	0.45	0.44	0.03	0.05	0.0248	0.142
Eosinophil (10^9/l)	0.13	0.12	0.1	0.03	0.02	0.5803	0.2882
Basophil (10^9/l)	0.02	0.02	0.02	0	0	0.2541	0.0622
Platelets (10^9/l)	267	251.5	234	3	9	0.2582	0.1415
Plasma Viscosity (ESR) (mm/h)	38	41	37.5	2	3	0.182	0.0351
Total T cell Count ($cm^{2)}$	1477	1541	1474.5	-63	38	0.6541	0.7079
CD4 count ($cm^{2)}$	374	361	334.5	-11	25.5	0.77	0.3435
CD8 count ($cm^{2)}$	1102	1036	1045	-47	-20	0.4774	0.5018
CD4/CD8 (ratio)	0.31	0.34	0.3	0.01	0.02	0.0749	0.0057

RBC Red cell count; MCV Mean corpuscular volume;
MCH Mean corpuscular Hemoglobin;
MCHC Mean corpuscular Hemoglobin concentration; RDW Red cell distribution width
WCC White cell count; $P<0.05$ Statistical significant difference.

Responders (n=36)

According to **Table 5.2** an average decrease of 89% ($CI_{95\%}$: 73%; 96%) of the individuals show decreased viral load counts with a median degree of change of 34% ($CI_{95\%}$: 73%; 96%) at 6 months and 85% [$CI_{95\%:}$ 68%; 94%] of the individuals show lower values with a median degree of change of ($CI_{95\%}$: 50%; 78.6%) at 12 months compared to baseline values.

Statistical significant changes occurred in the following variables coinciding with a decrease in viral load (see red highlighted figures in **Table 5.3 B**).

The **hematocrit** decreased from 0.41l/l to 0.39l/l (P=0.0316) from baseline to 12 months. The **MCHC** increased from 3.25g/dl to 33.0g/dl (P=0.0384) from baseline to 6 months and is unchanged after 12 months. The **RDW** decreased from 14.35 to 13.9 (P=0.0020) after 12 months. A decrease in the **monocyte** count from 0.49 $x10^9$/l to 0.44 $x10^9$/l (P=0.0248) is recorded after 6 months. **Plasma Viscosity (ESR)** is reduced from 38.0 mm/h to 37.5 mm/h (P=0.0351) after 12 months and the **CD4/CD8 ratio** decreased from 0.31 to 0.30 (P=0.0057) at 12 months.

5.4 DISCUSSION

The impact of the HIV on the human body is multi faceting, the virus affect the physiological function and the altered functions in turn causes further pathological changes. It is associated with an increase in renal, immune system, liver, cardiovascular and neurocognitive disorders. Haematological system disorders are common amongst HIV-infected individuals, the appearance are varied and prevail throughout the course of the disease. Anemia is a frequent complication of HIV infection (Mathews, Srivastava, Yadav & Sharma, 2013).

HIV infection has a negative influence on metabolism even in the early stages of the infection when no apparent clinical signs are visible (Szetela & Gasiorowski, 2010). The chronic inflammation caused by infection increases the metabolic needs of the

human body and poor absorption may lead to the depletion of essential nutrients resulting in a malnourished person with reduced immunity (Duggal, Chugh & Duggal, 2012).

The supplement used in the present investigation contained plant sterols, antioxidants and vitamins crucial to cell metabolism (Chapter 3 section 3.6). HIV infection results in activation of the immune system and local systemic oxidative stress. Although antioxidant protection is available in the body, it is proclaimed to be insufficient to contain the damage caused by reactive oxygen species (Coaccioli, Carpa, Fantera, Del Giorna, Standoli, Frongillo, Biondi & Puxeddu, 2010).

However any explanation pertaining to the exact mechanism involved due to the individual and/or synergistic role(s)/effect(s) of the ingredients on haematological and immunological function, do not fall within the realm/ambit of the present investigation.

Anemia is a well-known and frequent complication in HIV-infected individuals (Obinkorang & Yeboah, 2009; Marti-Carvajal, Sola, Pena-Marti & Comunian-Carrasco, 2011; Takuva, Maskew, Brennan, Sanne, MacPhail & Fox, 2013). Mathews and associates (2013) established that normocytic, normochromic anemia is prevalent in more than 40.1% of the HIV-infected individuals in their research study.

In this regard the present results show no significant alterations in **hemoglobin levels or red blood cell count** (see **Table 5.2**). The present results, however, show a decrease (p=0.0312) in **hematocrit** in 55% ($CI_{95\%}$38%; 71%) at six months and 63% ($CI_{95\%}$27%; 59%) at 12 months (Table 5.2). A decrease (p=0.0359) in the MCV is observed at 12 months. The reason(s) for these findings remain unexplained.

Plasma Viscosity (**Table 5.1**) is a useful marker indicative of inflammation in several conditions including HIV infection (Feldman, Aziz, Kang, Opondo, Belz & Sellers, 2013). The normal value for plasma viscosity is 3-12 mm/h. The plasma viscosity for the participants at baseline is 53.70 mm/h. The result of the high plasma viscosity caused rouleau formation of the red blood cells. In their study the participants show plasma viscosity levels of more than four times the normal value which is an indication of the level of inflammation present. In contrast, the present findings show a significant increase (p=0.0219) at 6 months but a significant decrease (p=0.0351) at 12 months in the responder group.

The **white cell count** (WCC**)** (**Table 5.1**) did not show significant changes during the investigation period. The more important component of the WCC is the lymphocyte count which includes the CD4 and CD8 cells. A lymphocyte count of <1 200 10^9/l (Cells) indicates an increase in disease progression and mortality (Sen, Vyas, Sanghi, Shanmuganandan, Gupta, Kapila, Praharaj, Kumar & Batra, 2011) In the present investigation the lymphocyte count decreased from 2.11 X 10^9/l to 1.90X 10^9/l over the 12 month period. This decrease is close (p=0.0563) to the 95% confidence interval level and is way above the critical count level specified by Sen *et al*. (2011).

The immune changes in HIV-infected individuals, including declines in the numbers as well as the ratio of **CD4** cell counts and **CD8** cell counts, are also found in malnourished individuals due to the negative effect on the thymus gland cell proliferation (Savin & Dardenne, 2010). Malnutrition causes thymic atrophy affecting immature CD8 and CD4 cells contributing to impaired peripheral immune response (Savin & Dardenne, 2010).

The pathogenicity of HIV is associated with its heterogeneity and is the end result of numerous abnormalities such as errors in enzyme reverse transcriptase and recombination at a rate of approximately 2% per kilobase per replication cycle results in selective immune pressure with negative affect on CD4 and CD8 cells. The reduction in CD4 and CD8 cells is contributed to oxidative cell activity

enhanced by insufficient nutrition (Momoh, Muhamed, Agboke, Akpabio & Osonwa, 2012).

The increase of HIV-RNA levels is associated with a decrease in T-cell count and function, as well as haematological variables such as hemoglobin concentration (Langford, Ananworanich & Cooper, 2007). Although the increasing risk of death in a malnourished HIV-infected individual is independent from the viral load and CD4 cell count (Grimble, 2009). Fauce, Yang and Effros (2007) reveal that the CD8 cell response is strategic in controlling HIV-replication and coincides with a strong anti-viral activity. Kim and associates (2010) state that the CD8 cells are vital to control viral replication by secreting interferon gamma that induces the production of antiviral proteins and immune response that destroys infected cells.

Pertaining to the present findings no statistical significant changes in CD4 and CD8 cell counts occurred in the entire study population (Table 5.1) or sub-populations (Table 5.3) over the entire duration of the trial period.

It is known that absolute CD4 cell counts *per se* to be inconsistent and may be misleading, for diagnostic purposes and the ratio of CD4 cell to CD8 cell count therefore to be considered a more reliable variable (Gaurav, Keerthilantha & Archna, 2011). Serrano-Villar, Gutiérrez, Vallejo, Hernandéz-Nova, Diaz, Fernandéz, Madrid, Dronda, Zamora, Munzo-Fernandéz, and Moreno (2013) state that an inverted **CD4/CD8 ratio** is associated with immune activation and increased disease progression and mortality regardless of the reduction of virus replication. The CD4/CD8 ratio is independently linked with T-cell activation even with long-term viral suppression (Serrano-Villar *et al.,* 2013). Furthermore, that lack of correlation even between a very small size of proviral DNA reservoir and T-cell activation in HIV-infected individuals with a continuous undetectable viral load for 10 years, are indicated (Poizot-Martin, Faucher, Obry-Roguet, Nicolino-Burnet, Ronot-Bregigeon, Dignat-George, & Tamalet, 2013).

Although CD4 and CD8 cell counts appears to be unaltered in the present investigation, a statistical significant decrease in the CD4/CD8 ratio (p=0.0048) is observed at 12 months. The only explanation for the decrease in the CD4/CD8 ratio could lie within the notion that a relatively small insignificant decrease in CD4 count in combination with a relatively small insignificant increase in the CD8 count in combination could significantly impact on the ratio and render a significant inverse relationship.

Research by Fauci, Pantaleo, Stanley and Weissman (1996) show that the **viral load** in HIV-infected individuals starts to rise after nine to 12 weeks after being infected and may increase to more than 10^8 RNA copies per milliliter plasma within 12 years. The plasma viral load of the HIV correlates with the rate of virus production in the lymphoreticular tissues. High levels of HIV copies in the plasma have been associated with rapid CD4 reduction and increased disease progression (Swanson, 1997).

The study of Oguntibeju (2004) reveals the short term effect of daily nutritional supplementation on the haematological variables, immune status and viral loads of HIV-infected individuals in the Free State province in South Africa. His results support the decrease in viral load that is indicated in the present study i.e. a statistical significant decrease load (p=0.0001) at both 6 months and 12 months following supplementation (see **Table 5.1**). The supplement taken by the HIV-infected individuals in this study contained several elements that combat oxidative stress and possibly contributed to the lower viral loads (See Chapter 3, section 3.3.2 for supplement content). Elements like zinc, magnesium and selenium inhibit intracellular HIV replication (Sepulveda & Watson, 2002) and possibly contributed towards the decrease in viral load.

The present results suggest that in terms of viral load and the number of individuals showing a decrease in viral load following use of the supplement, measurable

advantages in terms of clinical relevance that could not be ascribed/related to chance/coincidence, exist.

5.5 CONCLUSION

In terms of the primary aim of the present investigation the results suggest that a significant measurable decrease in viral load in HIV-infected individuals can be obtained by means of subjecting individuals to a nutritional fortification supplement strategy.

Due to the severity of the epidemic (in 2011, 10.6% (5.38 million) of the total population of the Republic of South Africa is HIV-infected) it seems possible that Southern Africa and particularly the rest of sub-Saharan Africa known to have limited access to quality nutritional recourses, could benefit from a well-balanced and carefully compiled nutritional supplement fortification regime but the compilation of the supplement could be specific for various specified regions.

Nutritional supplementation may delay the onset of AIDS and it furthermore, remains tempting to state that individuals receiving ARV could benefit from this practice as well.

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CHAPTER 6

INFLUENCE OF NUTRITIONAL SUPPLEMENT INTAKE ON PULMONARY FUNCTION IN HIV-INFECTED INDIVIDUALS LIVING IN THE MANGAUNG METROPOLITAN

ABSTRACT

The aim of the present investigation is to determine if a nutrient supplement (comprised of selected minerals, vitamins and herbs) administrated for a period of one year impact on pulmonary function in forty (40) HIV-infected individuals living in the Mangaung metropolitan.

Participants received the supplement with their monthly clinic visits for a period of 12 month. Blood samples were obtained, clinical assessment and pulmonary functions tests were performed during all monthly visits.

The main findings of the present investigation suggest that statistical significant changes in the median of the pulmonary function variables are evident for the PEF at six months and FEF_{75} at twelve months only: The significant increase (p=0.0302) in the median PEF from 6.09 l/sec to 6.62 l/sec coincides with a mean ($CI_{95\%)}$ of 68% (50%-81%) of the individuals showing a median (LQ - UQ) increase of 16.9% (11.5%;36.1%) in the Peak Expiratory Flow (PEF). The significant decrease (p=0.0484) in the median FEF_{75} from 1.35 l/sec to 1.17 l/sec coincides with a mean ($CI_{95\%)}$ of 70% (53%-83%) of the individuals showing a median (LQ - UQ) decrease of 28.1% (14%; 35.3%) in the $FEF_{75.}$after 12 month of exposure to the supplement. No statistical significant changes are observed for FVC, FEV_1, FEV_1/FVC and $FEF_{50.}$

The present results suggest that an improvement in air flow in the upper conductive airways of the lungs (trachea and bronchi) occurred after supplementation for six months but that the status deteriorated in the following six months. Airflow in the bronchioles (smaller airways) deteriorated at 12 months.

6.1 INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) dramatically reduced HIV related morbidity and mortality since 1990, resulting in HIV-infected people living much longer and even beyond middle age. The prolonged lifespan and reduced AIDS deaths created an interest in the long-term health of HIV-infected individuals (Mani, Haigentz & Aboulafia, 2012).

Early studies concluded that the lung is a major target for a variety of infections, immune defects and tumors in HIV-infected individuals (Mayaud & Cadranel, 2001). Pulmonary disease associated with HIV infection is an important co-morbidity with high mortality regardless effective treatment with HAART (Estébanez-Munoz, Soto-Abénades, Rios-Blanco & Arribas, 2012; Kynyk, Parsons, Para, Koletar, Diaz & Mastronarde, 2012).

The risk of lung cancer is greater in HIV-infected individuals compared with the non HIV-infected population (Silverberg, Choa, Leyden, Xu, Tang, Horberg, Klein, Quesenberry, Towner & Abrams, 2009). The incidence of lung cancer is two to four times higher in HIV-infected individuals than in the general population (Mani *et al.,* 2012). Lung cancer is currently the primary cause of non-AIDS related deaths in HIV-infected individuals, though the incidence of neoplasms such as non-Hodgkin lymphoma and Kaposi's sarcoma has decreased due to HAART (Estébanez-Munoz *et al.,* 2012). Histological, adenocarcinoma is most common in HIV-infected individuals (D'Jaen, Pantanowitz, Bower, Buskin, Neil, Greco, Cooly, Henry, Stem, Dezube, Stebbing & Abouiafia, 2010) followed by squamous-cel cancer (Engels, Brock, Chen, Hooker, Gillisom & Moore, 2006). HIV related factors that promote lung cancer may include the oncogenic properties of the HIV, recurrent respiratory infections, immunosuppression, reduced immune surveillance and increased susceptibility to carcinogens (Mani *et al.,* 2012).

To prevent the spread of pathogenic microbes into the pulmonary tract, the airways and lungs are protected first by the innate defense system encompassing the

epithelial cells, ciliary clearance, factors like antibacterial peptides and complement proteins. Cell support involves: macrophages, neutrophils, mast cells, natural killer cells, monocytes, and dendritic cells. The adaptive immune system is also involved with CD4 and CD6 cells prominent in both the airway and alveolar space (Brusselle, Joos & Bracke, 2011). With reduced immune protection opportunistic respiratory infections are associated with HIV-infected individuals (Estébanez-Munoz *et al.,* 2012).

Mycobacterial, bacterial and fungal, as well as viral lung infections are well described and persistent in HIV-infected individuals (Moja, Jalil, Perol, Quesnel, Cotte, Livrozet, Boibieux, Chamson, Vergnon, Lucht, Tran, Pozzetto & Genin, 1997; Rasheed & Thajuddin, 2011; Cunha, Syed & Hage, 2012). Tuberculosis (TB) and HIV and AIDS are closely linked. TB is the primary opportunistic infection amongst HIV-infected individuals (Raghavan, Alagarasu & Selvaraj, 2012). HIV is recognised as a risk factor for attracting TB disease. South Africa is the most affected country in the world where 73% of all diagnosed TB cases are co-infected with HIV (Daftary, 2012). Patients with TB experience anatomical lung changes secondary to the TB infection. It includes bronchiectasis, emphysematous as well as fibrotic changes. To measure these changes and impairment, pulmonary function tests are used. A present investigation by Vecino and co-workers found that the mentioned lung changes occur as early as 20 weeks after TB infection and that early diagnosis and treatment are important to prevent lung damage (Vecino, Pasipanodya, Slocum, Bae, Munguia, Miller, Fernandez, Drewyer & Weis, 2011).

Pulmonary function tests performed to measure lung impairment due to TB infection are: forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio and peak expiratory flow (PF) (Di Naso, Pereira, Schuh & Unis, 2011). Other organisms involved in respiratory infections in HIV-infected individuals include: *Pneumocystis jiroveci, Cryptococcus neoformans,* (Javier, Llovras, Santiago & Alcides, 2012), *Mycobacterium avian* (Bussone, Brossier, Roudiere, Bille, Sekkal, Charlier, Gilquin, Lanternier, Lecuit, Lortholary & Catherinot, 2012),

moulds like *aspergillus*, *zygomycetes* and *pseudallescheria* (Tammer, Tintelnot, Braun-Dullaeus, Mawrin, Scherlach, Schluter & Koning, 2011). Any human pathogenic organism may be the source of lung infection in HIV-infected individuals due to reduced immune protection (Rasheed *et al.,* 2011).

Obstructive lung disease includes chronic obstructive pulmonary disease (COPD), asthma and bronchitis (Hirani, Cavallazzi, Vasu, Pachinburavan, Kraft, Leiby, Short, Desimone, Squires, Weibel & Kane, 2011). COPD and asthma are on the increase in HIV-infected individuals. Recent studies demonstrate that 31% to 64% of HIV-infected individuals have pulmonary symptoms and that 6% to 21% presented with obstructive airway disease as measured with spirometry. Pre-bronchodilator and post-bronchodilator spirometry on HIV-infected individuals in an outpatient setting demonstrated that the pulmonary functions of 10% of the participants improved with 10%, suggesting that airway hypersensitivity might be a notable cause of airway obstruction in HIV-infected individuals (Gingo, Wenzel, Steele, Kessinger, Lucht, Lawther, Busch, Hillenbrand, Weinman, Slivka, McMahon, Zhang, Sciurba & Morris, 2012). Chest radiographic scoring by Desai and co-workers demonstrated chronic airway disease in HIV-infected individuals not linked to the usual chronic lung disease, suggesting that HIV infection does target the lungs independently (Desai, Copley, Barker, Elston, Miller, Wellsa, MInyati, Nathoo, Corbett & Ferrand, 2011).

Noninfectious lung conditions, especially emphysema, are appearing more frequently in HIV-infected individuals. Smoking is a contributing factor for increased COPD in HIV-infected individuals, as 40%-50% are current smokers. The prevalence of COPD among HIV-infected individuals who never smoked is also higher than individuals without HIV infection who are smoking (Hirani *et al.,* 2011). Additional predictive factors for the development of irreversible obstructive respiratory symptoms are: history of bacterial pneumonia, the use of intravenous drugs and HAART (Estébanez-Munoz *et al.,* 2012). HIV induced COPD contributes to skeletal muscle wasting which is recognised as an important extra pulmonary

manifestation of COPD contributing to increased morbidity and reduced quality of life (Ju & Chen, 2012).

An investigation by Gingo and co-workers (2012) determined that asthma is the most frequent diagnosed chronic pulmonary disease in HIV-infected individuals. Asthma associated symptoms are: wheezing, bronchodilator airway obstruction reversibility and increased medication use. Kynyk and co-workers (2012) found that HIV-infected individuals had increased respiratory symptoms, are more sensitive to bronchus-provocation and demonstrated elevated IgE which are indicative to asthma (Kynyk *et al.,* 2012). The inflammatory pathways leading to asthma related to T_H2-inflammation in HIV-infected individuals are cytokines IL-4, IL-5, and IL-13. In addition, the interaction between corticosteroids for the treatment of asthma and protease inhibitors used in some antiretroviral therapies may increase the peripheral corticosteroid levels.

Inhaled corticosteroids lessen the immune capability of the lungs to reduce airway inflammation, but increase the risk of pneumonia and TB infection (Calverley, Anderson, Celli, Ferguson, Jenkins, Jones, Yates & Vestbo, 2007). Siberry and co-workers (2012) established that HIV-infected individuals undergoing immune reconstruction with HAART resulting in immune activation may develop bronchial asthma after immune restoration (Siberry, Leister, Jacobson, Foster, Seage, Lipshultz, Paul, Purswani, Colin, Scott & Shearer, 2012).

The respiratory system is uniquely located to be constantly exposed to the external environment as well as the entire blood circulation of the human body (Kovach & Standiford, 2011). The diverse immune defects in HIV-infected individuals lead to a 10 times higher susceptibility to pulmonary infections compared to the general population (Rasheed *et al.,* 2011). Non-infectious lung conditions, such as emphysema, asthma (Hirani *et al.,* 2011) and lung cancer are becoming ever more prominent as HIV-infected individuals life span increases (Mani *et al.,* 2012). The compromised, deregulated immunity, and disrupted lymphocyte development of

HIV-infected individuals leading to pulmonary hypersensitivity need close scrutiny to avoid permanent lung damage (Siberry *et al.,* 2012).

In this chapter the focus is mainly based on spirometry (flow-volume curve measurements).

6.2. METHODOLOGY

Information based on the study design (see 3.1.1), study site (see 3.1.2), study population (see 3.2), intervention (see 3.3), pulmonary function testing (see 3.5.2.6) and analysis of data (see 3.6) was described in Chapter 3 as indicated.

6.3. RESULTS

This section reveals the demographic and relative results for two (2) populations categories. Baseline levels are obtained for all variables and compared to the various corresponding levels at 6 months and 12 months respectively.

6.3.1 Demographic results

A total of 132 HIV-infected individuals were enrolled but due to dropouts the study was completed with a final total of 40 individuals. The relatively high dropout figure can be ascribed to noncompliance with the protocol design i.e. not taking the supplement correctly or missing appointments–see Chapter 3: section 3.2. Seventeen (17%) of the 40 participants are male while 33 (83%) are female.

6.3.2. Relative results

Relative results refer to calculated values derived from specific measurements. The results obtained for two (2) populations are revealed i.e. entire study population (ESP) and a sub-population (SP).

6.3.2.1. Results for the entire Study Population category (ESP)

Table 6.1 indicates the normal values, mean, standard deviation, median and p-values for the specified pulmonary function variables at baseline, 6 and 12 months. Only statistical significant changes for a specified variable will be discussed. It is argued that if a statistically non-significant change is indicated, the researcher cannot form a scientifically validated opinion to make any statement pertaining to 'change' or 'difference' since these 'changes' or 'differences' could be related to 'chance'.

Table 6.1 Pulmonary function variables of the HIV- infected individuals at baseline, 6 months, and final visit at 12 months (n=40).

	BASELINE			6 MONTHS				12 MONTHS			
Flow volume curve	Mean	Std Dev	Median	Mean	Std Dev	Median	p Value	Mean	Std Dev	Median	p Value
FVC(l)	3.42	0.72	3.47	3.5	0.74	3.51	0.085	3.46	0.66	3.48	0.3928
FEV_1 (l)	2.83	0.68	2.97	2.88	0.62	2.93	0.3579	2.78	0.62	2.79	0.2026
FEV_1 /FVC (%)	81.79	8.71	81.34	82.38	6.39	82.9	0.6882	80.03	7.29	81.23	0.1335
PEF (l/sec)	6.14	1.97	6.09	6.76	1.6	6.62	0.0302	6.23	1.77	6.34	0.7479
FEF_{50} (l/sec)	3.76	1.26	3.79	3.72	1.21	3.7	0.777	3.5	1.23	3.37	0.0938
FEF_{75} (l/sec)	1.39	0.66	1.35	1.32	0.51	1.24	0.2773	1.22	0.58	1.17	0.0484

Std Dev	Standard deviation
FVC	Forced vital capacity
FEV	Forced expiratory volume in I second
FEV	FVC: Forced expiratory volume in 1 second / Forced vital capacity ratio
PEF	Peak expiratory flow
FEF_{50}	Forced expiratory flow at 50% of the total expired volume
FEF_{75}	Forced expiratory flow at 75% of the total expired volume
p≤0.05:	Variables demonstrate a statistical difference.
(l)	Litre
(l/sec)	Litre / second

The median Peak Expiratory Flow (PEF) demonstrated a statistical significant increase (p=0.0302) between baseline values and the values obtained at six months (see red figure in Table 6.1). The values of all other variables show no statistical significant changes (i.e. p>0.05) between baseline values and the values obtained at 6 months. The FEF_{75} (Forced expiratory flow at 75% of the total expired volume) show a significant reduction (p=0.0484) between baseline values and the values obtained at 12 months (see red figure in Table 6.1-bottom of the last

column). The values of all other variables show no statistical significant changes (i.e. p>0.05) between baseline values and the values obtained at 12 months.

6.3.2.2. Results for the Sub-Population category (SP)

The pulmonary function variables outlined in **Table 6.1** are subjected to an additional statistically analyses to express the mean percentage ($CI_{95\%}$ based on this mean) of *individuals* showing a median percentage change (LQ - UQ) for each and every variable (see **Table 6.2**).

Table 6.2. The percentage of HIV-infected individuals with change lung function variables, at baseline, 6 months, and 12 months (n=40)

VARIABLE	CHANGE	Number of participants	Degree of change	Number of participants	Degree of change
		0 to 6 months (%)	Median change (%) 0 to 6 months CI95%	0 to 12 months (%)	Median change (%) 0 to 12 months CI95%
FVC	Increase	60%	6.90%	52%	9.10%
		[43% ; 75%]	[3.3% ; 10.2%]	[36% ; 68%]	[3.0% ; 11.3%]
	Decrease	38%	5.80%	48%	4.80%
		[23% ; 54%]	[2.5% ; 8.8%]	[31% ; 64%]	[3.4% ; 7.6%]
	No change	2%	-	-	-
		[0.1% ; 15%]			
FEV_1	Increase	53%	4.30%	40%	4.90%
		[36% ; 68%]	[2.0% ; 8.4%]	[25% ; 57%]	[2.2% ; 9.2%]
	Decrease	40%	6.10%	55%	5.60%
		[25% ; 57%]	[2.1% ;11.1 %]	[38% ; 70%]	[2.9% ; 11.3%]
	No change	7%	-	5%	-
		[2% ; 21%]		[1% ; 18%]	
FEV1/FVC	Increase	37%	4.90%	27%	5.70%
		[23% ; 54%]	[1.2% ; 11.4%]	[15% ; 44%]	[0.6% ; 17.8%]
	Decrease	63%	3.30%	73%	4.40%
		[45% ; 77%]	[1.9% ; 5.2%]	[56% ; 85%]	[2.7% ; 6.5%]
	No change	-	-	-	-
PEF	Increase	68%	16.90%	50%	24.70%
		[50% ; 81%]	[11.5% ; 36.1%]	[34% ; 66%]	[15.7% ; 51.7%]
	Decrease	32%	13.70%	48%	22.60%
		[19% ; 49%]	[2.6% ; 21.1%]	[32% ; 64%]	[4.5% ; 30.8%]
	No change	-	-	2%	-
				[0.1% ; 15%]	
FEF_{50}	Increase	42%	14.10%	35%	15.00%
		[27% ; 59%]	[3.4% ; 41.5%]	[21% ; 52%]	[1.3% ; 81.3%]
	Decrease	58%	7.60%	65%	19.20%
		[41% ; 73%]	[8.0% ; 21.3%]	[48% ; 79%]	[16.1% ; 26.5%]
	No change	-	-	-	-
FEF_{75}	Increase	45%	21.00%	30%	21.00%
		[30% ; 61%]	[6.0% ; 46.3%]	[17% ; 47%]	[9.2% ; 79.3%]
	Decrease	55%	20.70%	70%	28.1
		[37% ; 70%]	[14.4% ; 25.4%]	[53% ; 83%]	[14.0% ; 35.3%]
	No change	-	-	-	-

At six months 68% (50%-81%) of the individuals show a median increase (p=0.0302) of 16.9% (11.5%; 36.1%) in the Peak expiratory flow (PEF). Over the period of twelve (12) months, 70% (53%-83%) of the individuals show a median (LQ - UQ) decrease (p=0.0484) of 28.1% (14%; 35.3%) in the FEF_{75} (see highlighted red figures in **Table 6.2).**

These two variables are the only variables where the changes measured could not be related to chance/coincidence.

6.4. DISCUSSION

Early studies concluded that the lung is a major target for a variety of infections, immune defects and tumors in HIV-infected individuals (Mayaud & Cadranel, 2001). Mycobacterial, bacterial and fungal, as well as viral lung infections are well described and persistent in HIV-infected individuals (Moja, *et al.,* 1997). In accordance, it is to be expected that deterioration in pulmonary function over a period of 12 months could be a possibility.

The main findings of the present investigation suggest that no statistical significant changes are observed for FVC, FEV_1, FEV_1/FVC and FEF_{50} over the period of 12 months when the supplement is used. Statistical significant changes in the median of the various pulmonary function variables are evident for the PEF at six months and FEF_{75} at twelve months only i.e. increase (P=0.0302) in the median PEF from 6.09 l/sec to 6.62 l/sec coincides with a mean ($CI_{95\%)}$ of 68% (50%-81%) of the individuals showing a median (LQ - UQ) increase of 16.9% (11.5%;36.1%) in the Peak Expiratory Flow (PEF) and the decrease (p=0.0484) in the median FEF_{75} from 1.35 l/sec to 1.17 l/sec coincides with a mean ($CI_{95\%)}$ of 70% (53%-83%) of the individuals showing a median (LQ - UQ) decrease of 28.1%(14%;35.3%) in the FEF_{75} after 12 months of exposure to the supplement is observed.

The PEF is the first part of air forcefully exhaled during maximal expiration following a maximal inspiration and represents/reflects the happenings in the upper conductive airways of the lungs (trachea and bronchi). The attempt is effort dependent with expiratory muscles and mechanism implicated (Enright, 2003). An improvement in the PEF could be due to an improvement in the function of expiratory muscles and mechanism implicated [HIV induced COPD contributes to skeletal muscle wasting which is recognized as an important extra pulmonary manifestation of COPD (Ju & Chen, 2012) and/or a decrease in air flow in the upper airways due to lumen diameter changes in the upper (trachea and bronchi) conductive airways brought about by chronic obstructive pulmonary disease (COPD), asthma and bronchitis (Hirani, *et al*., 2011).

The FEF_{75}, represents the air flow in the bronchioles (smaller airways) near end of maximal expiration (Ruppel, 2003) and a decrease in the FEF_{75} is indicative of the integrity of the bronchioles. In HIV-infected individuals a decrease in the FEF_{75} may be due to bronchiolitis (Gingo *et al.,* 2012) (at the time of measurement) or chronic bronchiolitis *per se* (Hirani, 2011).

6.5. CONCLUSION

In the first six (6) months individuals show an increase in the Peak expiratory flow (PEF). In accordance, it appears that improved function in terms of the happenings in the upper conductive airways of the lungs (trachea and bronchi) which are effort dependent with expiratory muscles and mechanism implicated, is evident.

At 12 months individuals show a decrease in the $FEF_{75.}$ In accordance it appears that the integrity of the bronchioles is tarnished. This could be due to lost in the elasticity of the lung parenchyma and/or swelling of the bronchioles transpiring in air trapping reflected by the decrease in the flow rate of air (FEF_{75}) in bronchioles i.e. small airways disease due to HIV progression.

These two variables are the only variables where the changes measured could not be related to chance/coincidence.

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CHAPTER 7

SUMMARY AND CONCLUDING REMARKS ON THE INTEGRATED RELATIONSHIP BETWEEN THE MAIN FINDINGS

7.1. INTRODUCTION

In 2011, 10.6% (5.38 million) of the total population of the Republic of South Africa was HIV-infected. Any disruption of the nutritional status of any individual will be detrimental to the ability of the immune system to protect the human body against infection. The immune system requires essential nutrients to produce needed defense mechanisms and defense cells (Carter, 2011). Not only will the inadequate intake of specific micronutrients suppresses the immune system (Wintergerst, Maggini & Hornig, 2007) but a reduction in immune system activity will also impact negatively on the nutritional status of the immune compromised individual (Afacan, Fjell & Hancock, 2012).

The importance of nutrient supplementation is highlighted by many HIV and AIDS programmes in sub-Saharan Africa that started to include nutritional assistance in addition to medical treatment to reduce malnutrition and improve the outcome in HIV-infected individuals (Tirivayi & Groot, 2011). The improvement of the nutritional status of HIV-infected individuals improves their physiological and psychological status with better quality of life (Oketch, Paterson, Maunder & Rollins, 2011).

7.2. PURPOSE

The purpose of this section of the Thesis is to integrate and align the main findings on baseline dietary intake, haematological status, immune status, viral load and pulmonary function following intake of a supplement. Furthermore, to state mutual tangent points (or differences) with the comparable findings of other researchers in this field of study.

7.3. MAIN FINDINGS OF THE PRESENT INVESTIGATION

The main findings on baseline dietary intake, haematological status, immune status and pulmonary function is stated and weighted.

7.3.1. Baseline dietary intake

The results of the present investigation show that the mean and/or median Energy (kJ) intake is higher than the specified Estimated Energy Requirement (EER) in both groups (males and females). The macronutrient (total protein, carbohydrate and fat) intakes in both males and females are higher than the Recommended Dietary Intake (RDA) and/or Adequate Intake (AI) specifications. Similar finding pertaining to higher energy intakes is reported by Oguntibeju, Van den Heever and Van Schalkwyk (2005) in a trial conducted over a period of 3 months in the same region (Mangaung Metropolitan).

The majority of the respondents consumed adequate amounts of micronutrients with the exceptions of *Vitamin D* and *Iodine.* The dietary intake of minerals and trace element met or exceeded the specified RDA or applicable Adequate Intake (AI) specifications. Research conducted in the Mangaung Metropolitan show that micronutrient deficiencies occurred in the majority of *HIV-infected children* in care centers (Steenkamp, Dannhauser, Walsh, Joubert & Veldman, 2009). The prominent deficient micronutrients were zinc, vitamin A, vitamin D and glutathione. These authors recommended vigorous macro- and micronutrient supplementation to eliminate these nutrient deficiencies.

7.3.2. Haematological variables

From all of the haematological variables the only statistical significant changes observed between baseline data and the data at 6 months are increases in the median ESR ($p=0.0219$) and MCHC ($p=0.0245$) coinciding with a decrease ($p=0.0001$) in viral load. At 12 months a statistical significant decrease in the median CD/CD8 ratio ($p<0.0048$), median Hematocrit concentration ($p<0.0312$), median MCV ($p<0.0359$), and median RDW ($p<0.0273$) accompanies a significant decrease in the median viral load ($p<0.0001$). The only variable(s) that show a statistical significant increase is the MCHC ($p<0.0003$) at 12 months.

A mean decrease of 89% ($CI_{95\%}$: 73%;96%) of the individuals show a decrease in viral load counts with a median percentage change of 34% ($CI_{95\%}$: 73%;96%) at 6 months. At 12 months a mean decrease of 85% [$CI_{95\%:}$ 68%; 94%] of the individuals show a decrease in viral load counts with a median percentage change of 62.9% ($CI_{95\%}$: 50%;78.6%) following the intake of the supplement.

Plasma Viscosity is a useful marker indicative of inflammation in several conditions including HIV infection (Feldman, Aziz, Kang, Opondo, Belz & Sellers, 2013). In their study the participants show plasma viscosity more than four times the normal value which is an indication of the level of inflammation present. In contrast, the present findings show a significant increase (p=0.0219) at 6 months but a significant decrease (p=0.0351) at 12 months in the responder group.

The **white cell count** of <1 200 10^9/l (Cells) indicates an increase in disease progression and mortality (Sen, Vyas, Sanghi, Shanmuganandan, Gupta, Kapila, Praharaj, Kumar, and Batra, 2011) In the present investigation the lymphocyte count decreased from 2.11 X 10^9/l to 1.90X 10^9/l over the 12 month period. This decrease is close (p=0.0563) to the 95% confidence interval level but is way above the critical count level specified by Sen *et al*. (2011).

The immune changes in HIV-infected individuals, including declines in the numbers as well as the ratio of **CD4** cell counts and **CD8** cell counts is evident (Savin & Dardenne, 2010). Malnutrition causes thymic atrophy affecting immature CD8 and CD4 cells contributing to impaired peripheral immune response (Savin & Dardenne, 2010). Pertaining to the present findings no statistical significant changes in CD4 and CD8 cell counts was observed for the entire study population or sub-populations during the entire trial period.

It is known that absolute CD4 cell counts *per se* to be inconsistent and may be misleading, for diagnostic purposes and the ratio of CD4 cell to CD8 cell count therefore to be considered a more reliable variable (Gaurav, Keerthilantha &

Archna, 2011; Serrano-Villar, Gutiérrez, Vallejo, Hernandéz-Nova, Diaz, Fernandéz, Madrid, Dronda, Zamora, Munzo-Fernandéz, Moreno, 2013). Lack of correlation even between a very small size of proviral DNA reservoir and T-cell activation in HIV-infected individuals (**CD4/CD8 ratio**) with a continuous undetectable viral load for 10 years, are indicated (Poizot-Martin, Faucher, Obry-Roguet, Nicolino-Burnet, Ronot-Bregigeon, Dignat-George & Tamalet, 2013). Although CD4 and CD8 cell counts appears to be unaltered in the present investigation, a statistical significant decrease in the CD4/CD8 ratio ($p=0.0048$) is observed at 12 months. The only explanation for the decrease in the CD4/CD8 ratio could lie within the notion that a relatively small insignificant decrease in CD4 count in combination with a relatively small insignificant increase in the CD8 count in combination could significantly impact on the ratio and render a significant inverse relationship.

The plasma **Viral load** in HIV-infected individuals starts to rise after nine to 12 weeks after being infected and may increase to more than 10^8 RNA copies per milliliter plasma within 12 years (Fauci, Pantaleo, Stanley & Weissman, 1996). High levels of HIV copies in the plasma have been associated with rapid CD4 reduction and increased disease progression (Swanson.1997). On the contrary, 89% ($CI_{95\%}$: 73%;96%) of the individuals show a decrease ($p=0.001$) in viral load counts with a median percentage change of 34% ($CI_{95\%}$: 73%;96%) at 6 months. At 12 months 85% [$CI_{95\%:}$ 68%; 94%] of the individuals show a decrease in viral load counts with a median percentage change of 62.9% ($CI_{95\%}$: 50%;78.6%) following the intake of the supplement. These findings suggest that in terms of viral load and the number of individuals showing a decrease in viral load following use of the supplement, measurable advantages in terms of clinical relevance exist and could not be ascribed to chance or be related to coincidence. A measureable decrease in viral load following supplementation was also observed in another trial with a different supplement, conducted by Oguntibeju (2004) in the same Metropolitan. In accordance it seems to be appropriate to state that supplementation can decrease viral load.

7.3.3. Pulmonary function

Pulmonary function test, particular spirometry is effective in diagnosing obstructive as well as restrictive respiratory diseases (Antwi, Gbekle, Cosmos, Ennin, Amedonu, Antwi-Boasiako, Clottey & Adzaku, 2011). Abnormally low forced expiratory flow rates frequently occur in HIV-infected individuals (O'Donnell, Bader, Zibrak, Jensen & Rose, 1988).

In the first six (6) months the only change observed for all variables is an increase in the Peak expiratory flow (PEF). In accordance, it appears that improved function in terms of the happenings in the upper conductive airways of the lungs (trachea and bronchi) which are effort dependent with expiratory muscles and mechanism implicated, is evident.

HIV-infected individuals commonly have symptoms of airway disease associated with lower forced expiratory air flow in one second (FEV_1) and forced mid expiratory flow (FEF_{50}) (Gelman, King, Neal, Pacht, Clanton & Diaz, 1999). The present results show no significant change in the $FEV_{1\ or}$ FEF_{50} At 12 months individuals showed a decrease in the $FEF_{75.}$ The FEF_{75}, represents the air flow in the bronchioles (smaller airways) near end of maximal expiration (Ruppel, 2003) and a decrease in the FEF_{75} is indicative of the integrity of the bronchioles. In HIV-infected individuals a decrease in the FEF_{75} may be due to bronchiolitis (Gingo, Wenzel, Steele, Kessinger, Lucht, Lawther, Busch, Hillenbrand, Weinman, Slivka, McMahon, Zhang, Sciurba & Morris, 2012) (at the time of measurement) or chronic bronchiolitis *per se* (Hirani, Cavallazzi, Vasu, Pachinburavan, Kraft, Leiby, Short, Desimone, Squires, Weibel & Kane, 2011). In accordance it appears that the integrity of the bronchioles is tarnished. This could be due to lost in the elasticity of the lung parenchyma and/or swelling of the bronchioles transpiring in air trapping reflected by the decrease in the flow rate of air (FEF_{75}) in bronchioles i.e. indicative of small airways disease. Small airways disease is frequently present in HIV-

positive individuals (Gelman *et al*., 1999). The damage to the lung tissue may be mediated by continuous immune activation and ongoing inflammation (Coffey, 2011).

7.4. INTEGRATED INTERACTIONS AND POSSIBLE CONSEQUENCES

The impact of the HIV on the human body is multi faceting, the virus affect the physiological function and the altered functions in turn causes further pathological changes. It is associated with an increase in renal, immune system, liver, cardiovascular and neurocognitive disorders (Meir-Shafrir & Pollack, 2012) Haematological system disorders are common amongst HIV-infected individuals, the appearance are varied and prevail throughout the course of the disease. Anemia is a frequent complication of HIV infection (Mathews, Srivastava, Yadav & Sharma, 2013).

7.4.1. Baseline dietary intake and anthropometric aspects

Significant weight loss and underweight are widely present among HIV-infected adults in sub-Saharan Africa. The nutritional status of the individual can impact on the BMI. In the present investigation the weight range for the group is considered to be normal weight (see Chapter 3: 3.5.1) i.e. a baseline median BMI of 24.5 and 24.0 after 12 months is observed and no statistical significant chance (p=0.6805) was calculated (see **APPENDIX F**). Poverty can impact not only on nutrient intake but also the type of food ingested. Furthermore, the impact of HIV-infection *per se*, can also impact on food intake.

Information on the EER, macronutrient and micronutrient intake was briefly discussed in section 3.1. No significant changes are observed for any of the anthropometric parameters i.e. BMI; and circumferences [indicative of health and nutritional status such as malnutrition and/or progression of the syndrome/disease *per se* (Gibson, 1998; Lee & Nieman, 2010). These findings were not discussed but the findings are stated in **APPENDIX F**. In accordance, it could be possible that the

relatively high energy intake (Carter, 2011) and/or a relatively high protein intake (after Kim, Rimm & Gorbach, 2001; Oguntibeju, 2004) may have prevented the phenomena surrounding muscle wastage. This view, however, should be appreciated within the notion put forward by Ivers, Cullen, Freedberg, Block, Coates and Webb (2009) i.e. that HIV and AIDS affect the metabolism of insulin and glucagon leading to muscle wasting. A decrease in insulin could impact on anabolic growth and catabolic (energy yielding) processes. A decrease in glucagon could negatively impact on plasma blood glucose levels.

7.4.2. Baseline dietary intake and immunological aspects

The majority of the respondents consumed adequate amounts of micronutrients with the exceptions of *Vitamin D* and *Iodine.*

7.4.2.1. Vitamin D deficiency and immune function

Vitamin D deficiency could impact on homeostasis in many ways: Lagishetty, Lui & Hewison (2011) show that vitamin D is involved in the regulation of the innate immune system (see Chapter 2: 2.6.1.1): Toll-like receptors (TLRs) of monocytes and macrophages plays an important role in innate immune response (Lagishetty, Lui & Hewison. 2011). The stimulation of the TLRs in macrophages by anti-microbial peptides results in the conversion of vitamin D to its active form. The role of vitamin D is to assist with the induction of cathelicidin (protein with c-terminal cationic anti-microbial domain) to be released at the site of infection (Beard, Bearden & Striker, 2011; Verrusio, Linguanti, Di Maio, Martinez, Marigliano & Cacciafesta, 2012).

Vitamin D, a fat soluble pre-hormone acts via metabolites like1,25$(OH)_2$D that binds to the nuclear vitamin D receptor (Gueli, Verrusio, Linguanti, Di Maio, Martinez, Marigliano & Cacciafesta, 2012) and suppressed T-helper (Th_1) CD4 cells to enhance Th_2 CD4 cells production. Vitamin D-activating enzyme (CYP27B1) assists in linking monocytes and pathogen sensing mechanisms.

It has already been noted that in people living with HIV and AIDS, lifestyle, hormonal factors, decreased physical activity, lower intake of calcium and vitamin *D* as well as lower plasma calcium levels, all contribute to the progression of the disease (Borderi, Gibellini, Vescini, De Crignis, Cimatti, Biagetti & Tampellini, 2009; Campa & Baum, 2010). The present findings show that the mean and median intake for Calcium is relatively higher than the specified RDA of 1,000 mg/day for both genders, but Calcium intake did not exceed the upper limit of 2500mg per day (Curtiss & Johnson, 2007) and raises no concern. However, a link between vitamin D intake and calcium absorption from the alimentary tract exists: Calcium is better absorbed when taken in combination with Vitamin D. Lack of the simultaneous intake of calcium and Vitamin D intake in the diet impedes on the formation of the hormone calcitriol (known as the “active vitamin D”). This in turn leads to insufficient calcium absorption from the diet. In this situation, the body must take calcium from its stores in the skeleton, which weakens existing bone and prevents the formation of strong, new bone. Although the present results show sufficient intake of Calcium, a decrease in Vitamin D intake is observed and, accordingly, could impact on the health status of the individuals with low Vitamin D intake.

7.4.2.2. Iodine deficiency and immune function

Iodine is required in the synthesis of thyroid hormones, triiodothyronine (T3) and thyroxin (T4). Thyroid hormones play a major role in the metabolic processes of the body. It is involved in the regulation of macronutrient metabolism and basal metabolic rate. Iodine deficiency may lead to low levels of thyroid hormones resulting in full-blown hypothyroid syndrome (myxedema). Symptoms include mental sluggishness, edema and low metabolic rate (Marieb & Hoehan, 2013).

The high concentration of iodine in the thymus supports the importance of this element in the immune system. Leukocyte myeloperoxidase enzymes in cell-mediated immunity (See Chapter 2: 2.6.1.2) use iodine to produce iodine-free radicals. Iodine also increases immunoglobulin-G production in human lymphocytes. The non-endocrine effects of iodine include increase in the movement

of granulocytes into areas of inflammation and improve phagocytosis ability of the granulocytes.

The immune deficiency in iodine-deficient individuals may be restored by oral administration of iodine (Venturi, 2009). According to Beltran, Lescure, El Esper, Schmit and Desailloud (2006), iodine deficiency in HIV-infected individuals may contribute towards hypothyroidism.

7.4.2.3. Vitamin D deficiency and pulmonary function

Growing evidence exist that Vitamin D (especially vitamins D_2 and D_3) are involved in respiratory infections and immune function in asthma due to its immunomodulatory function (Huang, Porpodis, Zarogoulidis, Domvri, Giouleka, Papaiwannou, Primikyri, Mylonaki, Spyratos, Hohenforst-Schmidt, Kioumis & Zarogoulidis. 2013). Vitamin D modifies the airway responsiveness by reducing airway inflammation (Rance, 2013). In accordance, the reduced intake of vitamin D may contribute to the reduction in the integrity of the smaller airways.

Supplementation with specific micronutrients like vitamin D will benefit HIV-infected individuals in particular to combat cardiovascular events (Choi, Lo, Mulligan, Schnell, Kalapus, Li, Hunt, Martin, Deeks, & Hsue, 2011) and restoring the immune homeostasis. Vitamin D supplementation according to the U.S. food and drug administration should not be more than 2000 IU daily due to toxicity of this fat-soluble vitamin (Makariou, Liberopoulos, Elisaf & Challa 2011; Van Schoor & Lips, 2011). Nutritional addition of Beta-sitosterol (naturally found in peanuts, sesame seeds and soybeans) supports the action of vitamin D to improve macrophage function. Macrophages produce cytokines and nitric oxide, an important defense mechanism against infection. Dietary supplementation of vitamin D as an anti-inflammatory nutrient may improve the immune function and the pulmonary function of the body (Ng, Niti, Yap, & Tan. 2013).

7.4.3. Baseline dietary intake and haematological aspects

Anemia is a well-known and frequent complication in HIV-infected individuals (Takuva, Maskew, Brennan, Sanne, MacPhail, & Fox. 2013; Mathews *et al*., 2013). Hemoglobin values are associated with reduced intake of protein and essential nutrients in HIV-infected individuals. This and other factors such as change in cytokine production and decreased erythropoietin concentration lead to anemia associated with the progression of AIDS and shorter survival of HIV-infected individuals. Mathews and associates (2013) established that normocytic normochromic anemia is prevalent in more than 40.1% of the HIV-infected individuals in their research study. This may explain why the reduction in median hemoglobin value in this- investigation showed no statistical significant alterations.

In this regard the present results show no significant alterations in hemamaglobin levels or red blood cell count (see **Table 5.2**). The present results show a decrease (p=0.0312) in hematocrit in 55% ($CI_{95\%}$38%; 71%) of the participants at six months and 63% ($CI_{95\%}$27%; 59%) of the participants at 12 months.

7.4.4. Viral load

Viral load in HIV-infected individuals starts to rise after nine to 12 weeks after being infected and may increase to more than 10^8 RNA copies per milliliter plasma within 12 years (Fauci, *et al.,* 1996). It also appears that the plasma viral load of the HIV correlates with the rate of virus production in the lymphoreticular tissues and is associated with rapid CD4 reduction and increased disease progression (Swanson.1997). On the contrary, 89% ($CI_{95\%}$: 73%; 96%) of the individuals show a decrease (p=0.001) in viral load counts with a median percentage change of 34% ($CI_{95\%}$: 73%;96%) at 6 months. At 12 months 85% [$CI_{95\%:}$ 68%; 94%] of the individuals show a decrease in viral load counts with a median percentage change of 62.9% ($CI_{95\%}$: 50%;78.6%) following the intake of the supplement. These findings suggest that in terms of viral load and the number of individuals showing a decrease in viral load following use of the supplement, measurable advantages in terms of

clinical relevance exist and could not be ascribed to chance or be related to coincidence. A measureable decrease in viral load following supplementation was also observed in another trial conducted by Oguntibeju (2004) in the same Metropolitan. In accordance it seems to be appropriate to state that supplementation can decrease viral load.

The increase of HIV-RNA levels is associated with a decrease in T-cell count and function, as well as haematological variables such as hemoglobin concentration (Langford, Ananworanich & Cooper, 2007). The present results suggest that an inverse relationship between these variables also seems to exist i.e. decrease in viral load coincides with no alterations in T-cell count and hemoglobin concentration.

Although the increasing risk of death in a malnourished HIV-infected individual is independent from the viral load and CD4 cell count (Grimble, 2009), other researchers state that the CD8 cells are vital to control viral replication by secreting interferon gamma that induces the production of antiviral proteins and immune response that destroys infected cells (Kim, Lee, Hong & Kim, 2010). It is known that absolute CD4 cell counts *per se* to be inconsistent and may be misleading, for diagnostic purposes and the ratio of CD4 cell to CD8 cell count therefore to be considered a more reliable variable (Gaurav, *et al.,* 2011). Although CD4 and CD8 cell counts appears to be unaltered in the present investigation, a statistical significant decrease in the CD4/CD8 ratio ($p=0.0048$) is observed at 12 months. The only explanation for the decrease in the CD4/CD8 ratio could lie within the notion that a relatively small insignificant decrease in CD4 count in combination with a relatively small insignificant increase in the CD8 count in combination could significantly impact on the ratio and render a significant inverse relationship. Furthermore, it appears that the CD4/CD8 ratio is independently linked with T-cell activation even with long-term viral suppression (Serrano-Villar *et al.,* 2013).

7.4.5. Supplement

The researcher had no indication or any insight knowledge on the baseline dietary intake levels of the 40 HIV-infected individuals prior to the formulation of the product. Furthermore, the researcher did not participate in formulating the product and viewed the formulation as being an arbitrary concoction of 'nutritional substances' where claims are made in terms of the 'beneficial effects' ascribed to use of the supplement by HIV-infected individuals.

The primary goal of the present investigation was to evaluate if the supplement impact on viral load and gather information pertaining to various and numerous variables indicative of the health status of HIV-infected individuals (see chapter 5).

The ingredients and their respective quantities that comprise the product are revealed in Chapter 3: section 3.3.2. The addition of Spirulina to the mixture does not reflect the actual amounts of specific additional essential fatty acids, beta-carotene, minerals, vitamins and high quality protein added to the formulation as specified in the formulation. However, Spirulina *per se* potentiates the immune system, suppressing cancer development and viral infection (Watanuki, Ota, Malina, Tassakka, Kato & Sakai, 2006). The same argument could be put forward for the non-specified compositions for other substances (Biociden and Colostrum) specified in the formulation.

In retrospect/hindsight, and at this point in time, it appears to be easy to predict what the possible outcome in terms of the presence of the micronutrient components of formulation should entail i.e. the present results show a decrease in the intake of Vitamin D and Iodine and should have been present (not absent) in the formulation. In terms of baseline dietary intake, all other micronutrients on average (mean and median) met or exceeded the RDA/AI and one could consider supplementation as being an unnecessary dietary additive. In terms of the findings pertaining to the viral load, the venture, however, holds water.

Research conducted in the Mangaung Metropolitan show that micronutrient deficiencies occurred in the majority of *HIV-infected children* in care centers (Steenkamp *et al.,* 2009). The prominent deficient micronutrients were zinc, vitamin A, vitamin D and glutathione. These authors recommended vigorous macro- and micronutrient supplementation to eliminate these nutrient deficiencies.

The study of Oguntibeju (2004) reveals the short term effect of daily nutritional supplementation on the Haematological variables, immune status and viral loads of HIV-infected individuals in the Free State province in South Africa. His results support the decrease in viral load that is show in the present study i.e. a statistical significant decrease load (p=0.0001) at both 6 months and 12 months following supplementation.

The supplement taken by the HIV-infected individuals in this study contained several elements that combat oxidative stress (although not tested in this study) and possibly contributed to the lower viral loads (see Chapter 3 section 3.6.1 for supplement content). Elements like zinc, magnesium and selenium inhibit intracellular HIV replication (Sepulveda & Watson. 2002) and possibly contributed towards the decrease in viral load. The exact mechanisms involved, however, remains unresolved.

7.5. LIMITATIONS OF THE PRESENT INVESTIGATION AND RECOMMENDATIONS

- It could be argued that although the stage of disease progression was known when the participants enrolled, the study population is comprised of individuals covering a broad spectrum of diverse immune-respons states. The diversity in the immune-respons states between and amongst individuals transpires in relatively large standard deviations for numerous variables in the study population. Although statistical significant changes

with corresponding confidence intervals for numerous specific variables were noted, the final sample size (n = 40) could be rendered insufficient if relatively small changes pertaining to any specified variable coincide with large standard deviations. To combat this constraint sample size must increase. The relatively small sample size was mainly brought about by the unpredicted high dropout numbers/rates (i.e. 40/132). This could have affected the statistical power of the present findings where small changes occurred.

- The present findings cannot be extrapolated to be representative of similar results being obtained in the population of all HIV-infected individuals in any other regions/country elsewhere. The results only relate to the present specified study population in the Mangaung Metropolitan. In accordance, baseline dietary intake information for example, should be determined for various regions/countries in order to formulate the composition of a specific supplement for that region/country.

- The plasma levels for all comparable substances pertaining to measurable baseline dietary intake levels of micronutrients should be determined. This recommendation could give information on the absorption rates and fractions in terms of the Baseline dietary intake levels;

- The present findings relate to an intervention where a nutritional supplement comprised of vitamins, minerals and herbs is administered to HIV-infected individuals. No clarity exists to state which substance or combination of substances that comprise the product is responsible for the observed changes in the variables stated.

- CD4 counts, CD8 counts and the CD4:CD8 ratio play major roles in immunity. However, no statistical significant changes was observed for these variables except for the statistical significant decrease in the CD4/CD8

ratio (p=0.0048) observed at 12 months. Cell counts says something but not all. It is recommended that cell activity levels for these specified cell counts should also be determined in order to elaborate on the immune status in a holistic manner.

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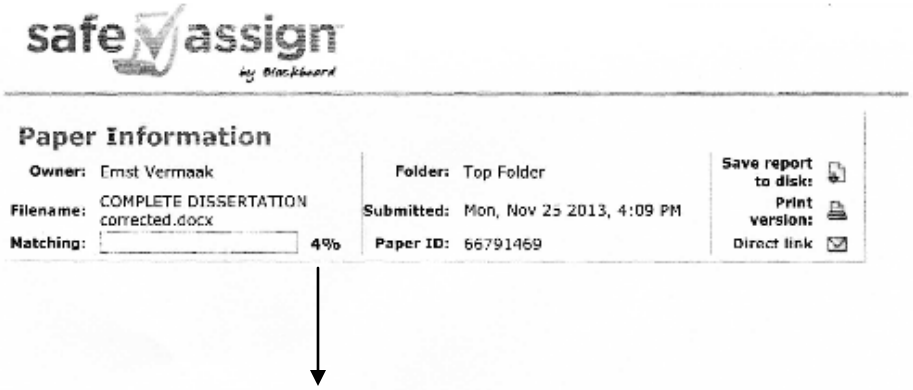
APPENDICES

APPENDIX A



Citations (4%) indicated for the reference lists

APPENDIX B

HIV/AIDS Nutrition Project

Date: ----------

CONSENT TO PARTICIPATE IN RESEARCH

You have been asked to participate in a research study.

You have been informed about the study by …… .

You may contact Prof E van den Heever-Kriek at 082 770 5356 any time if you have questions about the research or if you are injured as a result of the research.

You may contact the Secretariat of the Ethics Committee of the Faculty of Health Sciences, UFS at telephone number (051) 4052812 if you have questions about your rights as a research subject.

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to terminate participation.

If you agree to participate, you will be given a signed copy of this document as well as the participant information sheet, which is a written summary of the research.

The research study, including the above information has been verbally described to me.
I understand what my involvement in this study means. I also understand that my patient information will be regarded as confidential, that my participation is voluntary and that I could withdraw at any time.

_____ _____

Signature of Participant Date

_____ _____

Signature of Translator Date

INFORMATION DOCUMENT

STUDY TITLE: The influence of a liquid nutritional supplement on the immune– and health status of HIV-positive/AIDS patients in the Bloemfontein region.

Dear participant, we are inviting you to be part of this clinical trail and would be very thankful if you could see your way open to be part of this study. Below you will find all the information about this study and your responsibilities as participant.

INTRODUCTION

Several vitamins and minerals are critical for fighting HIV infection because they are required by the immune system and major organs to attack infectious pathogens. Research indicates that in the early period of HIV infection, weight gain or maintenance might be achieved through nutrition. Data on the prevalence of malnutrition, dietary intake and / or supplementation in HIV-infected persons in industrialized countries, is widely available. However, this information is often scarce in Africa where endemic malnutrition and lack of nutrition management are common. Therefore, study to evaluate the role of nutritional supplementation in HIV-positive patients becomes necessary, especially in a developing country such as South Africa. This clinical trail is set up to investigate the efficiency of a liquid nutritional supplement on the immune system of HIV/AIDS patients with a suppressed immune system.

STUDY DESIGN

This study will be divided into 4 phases:

1 Initial phase
2 Screening/baseline visit
3 Monthly visits
4 Final visit

During the initial phase of the project written approval for the protocol will be obtained from the Ethics Committee of the Faculty of Health Sciences, University of the Free State, Bloemfontein, before the first administration of study supplement. The training of staff and field workers will also take place during this phase.

The rest of the study consists of a screening visit and monthly visits over a period of one year. The first monthly visit will be scheduled seven days after screening. Visit two will be scheduled thirty days after visit one, etc. A deviation of –2 and +2 days from schedule visits will be allowed. Figure 1 below is a summary of the study visits and data collection of the project.

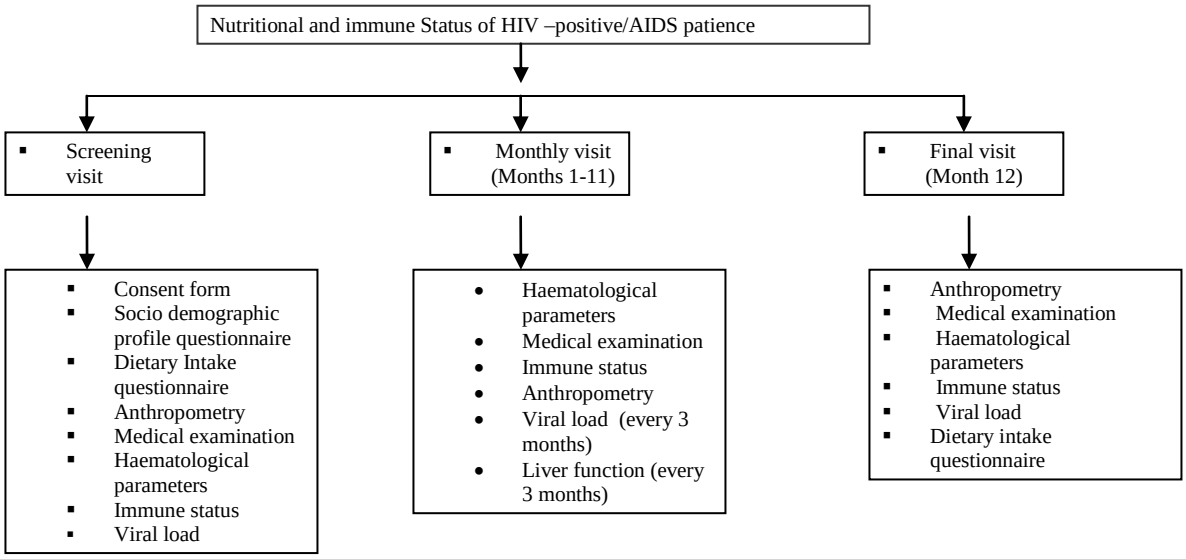


Figure1. A summary of the study visits and data collection of the project.

Visit Procedures

Respondents will be transported to the Medi Inn Centre monthly. The respondents will sign the consent form at their first visit. Researchers will interview the respondents to determine the demographic profile and food consumption patterns of the respondents. Compliance will also be measured at the end of the study, using a standardized questionnaire.

The researchers will perform Anthropometric- and baseline biochemical measurements. Anthropometric measurements will include: Bodyweight, height, hip circumference, waist circumference, percentage fat, skin-fold thickness, and BMI.

Registered medical personnel will draw blood specimen from patients before, during and after supplementation for a period of one year. Blood samples will be collected during the screening and monthly visits by registered medical personnel and send to Pathcare (Pathology laboratory) for the analysis of the immune status, health status, viral load (very 3 months), haematological parameters and liver functions (every 3 months).

STUDY POPULATION

Fifty volunteers living with HIV will be selected after the screening visit, who fulfilled the inclusion criteria, did not meet any of the exclusion criteria, and who gave written informed consent, will be entered into the study.

Inclusion Criteria

- Male and female subjects from 18 to 65 years of age that are HIV/AIDS positive.
- $CD4^{+}$T-cell counts 200 – 350 cells/mm^3

- Findings within the range of clinical acceptability in medical history and physical examination, and laboratory results acknowledged by the clinical investigator.
- Willingness to undergo a pre-study physical examination and pre- and post study laboratory investigations.
- Ability to comprehend and willingness to sign the statement of informed consent.

Withdrawal criteria
Subjects have the right to withdraw from the study at any time, irrespective of the reason, without detriment of their medical care. These patients will be handled as drop-outs. Drop-outs to whom the study supplement was administrated will be requested to undergo the post-study evaluation.
The following may also lead to withdrawal:
6.3.2.1 Adverse events as a result of taking the study supplement, at the discretion of the clinical investigator.
6.3.2.2 Protocol violation by subjects, at the discretion of the clinical investigation.

Subject identification
- Each enrolled subject will receive a number (01 – 50) and will retain this number throughout the study.
- Each enrolled subject will retain his initials obtained from a copy of his identification book (i.e. three digitals)
- Each enrolled subject will be identified by date of birth (six digits).

Participation is voluntary, and refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled; the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Confidentiality
Efforts will be made to keep personal information confidential. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law.
Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Ethics Committee for Medical Research and the Medicines Control Council.

Restrictions on patients
Medicines:
All concomitant medication will be documented in CRF. If any additional medication is required during the study period, the clinical investigator has to be notified. Concomitant medication must state generic and trade names, strength, frequency and duration of treatment.
Diet:
The patients must carry on with their normal diet.

Safety of the patients
The project is very safe. The patients will be monitored daily during the intake of their supplement. The supplement is a liquid multi vitamin. During each visit, physical and medical examinations will be carried out on the patients by a clinical physician. Blood samples will be drawn from the patients to determine their immune- and health status and

every 3 months their viral load and liver function. Registered medical personnel will draw blood specimen from patients before, during and after supplementation for a period of one year.

Financial implication for the patients
There is no financial implication for the patients. The patients do not have to pay for their medical consultations or any of their clinical results. They will receive treatment for free. They will also receive the supplement freely. During each visit they will also receive a meal and free transport to the clinic.
The patients will not be paid for been part of the trail.

STUDY SUPPLEMENT
Supplement Ingredients

Below is a summary of the ingredients of the multi-vitamin/anti-oxidant supplement what will be used during trail.

Supplementation
The patients will receive 1 pill once daily (between 07:00 - 09:00). Staff members of the South African Red Cross Community Home Based Care Program, in the community, will handle the dosing and monitoring of the supplement intake and compliance on daily basis. Patients must carry on with their normal diet.

Contact details of researcher(s) – Prof E van den Heever-Kriek (Project leader)
Cell nr: 082 770 5356
Mr. E Vermaak (Researcher)
Cell nr: 082 775 6261
Dr. P Basson (Researcher)
083 414 8445

Contact details of REC Secretariat and Chair – for reporting of complaints/problems.
Phone nr: 051-405 2812

Moralo wa phepo wa HIV/AIDS

Letsatsi: ----------

TUMELLO YA HO NKA KAROLO DIPATLISISONG

O kopilwe ho nka karolo thutong ya dipatlisiso.

O ile wa tsebiswa ka thuto ena ke …….. .

O ka nna wa ikopanya le Prof E van den Heever-Kriek nomorong ya 082 770 5356 nako e nngwe le e nngwe ebang o nale dipotso mabapi le dipatlisiso kapa ebang o ka tswa kotsi ka baka la dipatlisiso.

O ka nna wa ikopanya le Mongodi wa Ethics Committee ya Faculty of Health Sciences, UFS nomorong ya mohala ya (051) 4052812 ebang o nale dipotso ka ditokelo tsa hao jwaloka eo ho etswang dipatlisiso ka yena.

Ho nka karolo ha hao dipatlisisong tsena ke boithaopong ba hao, mme o keke wa fumantshwa kotlo kapa wa lahlehelwa ke menyetla ya hao ebang o ka hana kapa wa nka qeto ya ho kgaotsa ka ho nka karolo.

Ha o dumela ho nka karolo, o tla nehwa khopi e saennweng ya tokomane ena hammoho le leqhephe la ba nkang karolo e leng le ngotsweng kgutsufatso ya dipatlisiso.

Thuto ya dipatlisiso ho kenyellwa lesedi le ngotsweng ka hodimo, di ile tsa hlaloswa ho nna ka molomo.

Ke utlwisisa hore ho nka karolo ha ka thutong ena ho bolelang. Ke boetse ke utlwisisa hore tlhahiso leseding e mabapi le dintlha tse amang botho ba ka, e tla nkwa e le sephiri le hore ho nka karolo ha ka ke boithaopo le hore nka nna ka ikgula nako e nngwe le e nngwe.

_

_____	_____
Tshaeno ya motho ya nkang karolo	Letsatsi
_____	_____
Tshaeno ya mofetoledi	Letsatsi

TOKOMANE YA TLHAHISO LESEDING

SEHLOOHO SA THUTO: Thunthetso ya phepo ya mokedikedi wa tlatsetso mabapi le twantsho ya kokwanahloko le bophelo bo botle ho boemo ba bakudi ba HIV/AIDS lebatoweng la Bloemfontein.

Monkakarolo ya ratehang, re o mema hore o be karolo ya teko ena ya bongaka mme re tla leboha haholo ebang o ka ipha sebaka sa ho ba karolo ya thuto ena. Ka tlase, o tla fumana lesedi kaofela mabapi le thuto ena le maikarabelo a hao jwaloka monkakarolo.

SELELEKELA

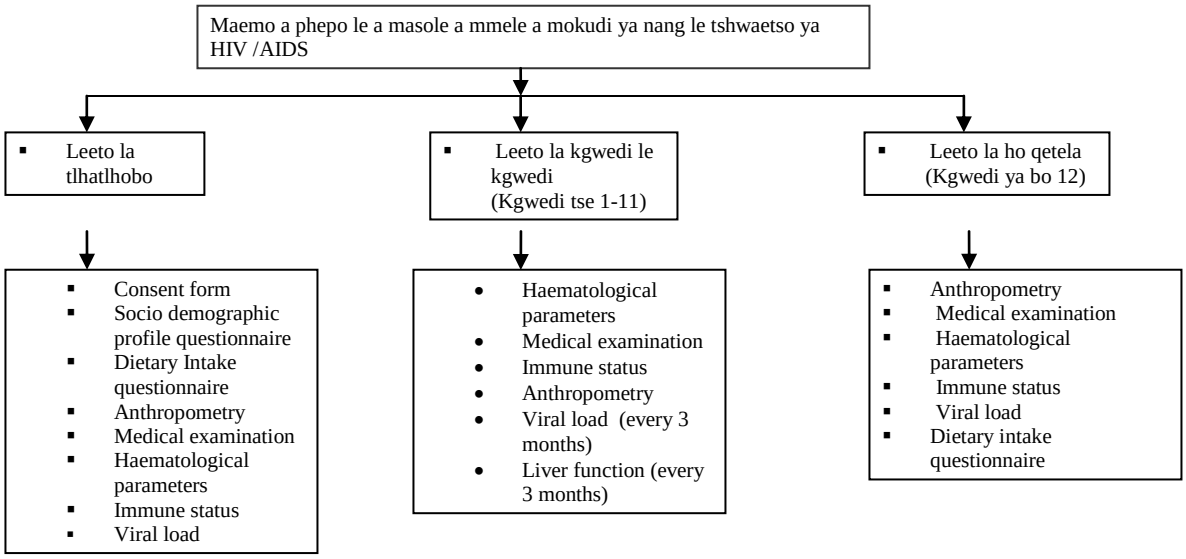
Divithamini le dimenerale tse ngata ke ntho tsa bohlokwa twantshong ya tshwaetso ya HIV hobane di batlwa ke dithibela kokwanahloko le ditho tsa bohlokwa tsa mmele mabapi le ho hlasela kokwanahloko tse tshwaetsang. Dipatlisiso di bontsha hore qalong ya tshwaetso ya HIV keketseho ya boima ba mmele kapa ntshetsopele ya boima boo, di ka fihlellwa ka phepo. Tlhahisoleseding mabapi le ho ata ha phepo e mpe, dijo tse jewang le disebediswa tsa tlatsetso ho lwantshwa kokwanahloko ho batho ba nang le tshwaetso ya HIV dinaheng tse atlehileng moruong ke ntho e fumanehang ka bongata. Leha ho le jwalo, tlhahisoleseding ena ke ntho e haellang ka hara Afrika moo phepo e mpe hammoho le kgaello ya tsamaiso e ntle ya phepo di atileng teng. Ka tsela eo, thuto mabapi le ho hlahloba karolo ya tlatsetso phepong ya bakudi ba nang le tshwaetso ya HIV e ba ntho e hlokahalang haholo naheng e ntseng e tutuha jwaloka Afrika Borwa. Tlhatlhobo ena ya teko ya bongaka e etseditswe ho fuputsa molemo wa phepo ka mokedikedi masoleng a mmele a bakudi ba nang le HIV/AIDS bao e leng hore masole a mmele ya bona ha a sebetse.

SEBOPEHO SA THUTO
Thuto ena e tla arolwa ka mekgahlelo e mene:

5 Mokgahlelo wa ho qala
6 Tlhatlhobo/leeto la motheo
7 Maeto a kgwedi le kgwedi
8 Leeto la ho qetela

Nakong ya mokgahlelo wa pele wa porojeke ho tla fumanwa kananelo e ngotsweng fatshe ho tswa Ethics Committee of the Faculty of Heatlh Sciences ya Yunivesithi ya Foreistata, Bloemfontein, e mabapi le metjha e salwang morao pele ho sebediswa teko ya pele thutong. Ke mokgahlelong ona moo basebetsi ba tla fumantshwang thupello ya bona.

Boholo ba thuto bo kenyelletsa maeto a tlhatlhobo le maeto a kgwedi le kgwedi nakong e ka etsang selemo se le seng. Leeto la pele la kgwedi le kgwedi le tla hlophiswa matsatsing a supileng kamora tlhatlhobo. Leeto la bobedi le tla hlophiswa matsatsi a mashome a mararo kamora ketelo ya pele, jj. Ho tla dumellwa mokgelo wa matsatsi a -2 le +2 tlhopisong ya maeto. Setshwantsho sa 1 se ka tlase ke kakaretso ya maeto a thuto le pokelletso ya tlhahisoleseding ya porojeke.



Setshwantsho sa 1. Kakaretso ya maeto a thuto le pokelletso ya tlhahisoleseding ya porojeke.

Tsamaiso ya maeto

Baarabi ba dipotso ba tla iswa Medi Clinic ka dipalangwang kgwedi le kgwedi. Baarabi ba dipotso ba tla saena foromo ya tumello ketelong ya bona ya pele. Babatlisisi ba tla buisana le baarabi ho netefatsa dintlha mabapi le morabe le tlwaelo e mabapi le dijo tseo ba di jang. Qetellong ya thuto, ho tla methwa taba ya hore na ba phethahatsa ka moo ho batlwang ka teng ka ho sebedisa lenane la dipotso.

Babatlisisi ba tla sebedisa mokgwa wa ho lekanyetsa wa Anthropometric le baseline biochemical. Mokgwa wa ho lekanyetsa wa Anthropometric o tla kenyelletsa: Boima ba mmele, bophahamo, bophara ba letheka, diphesente tsa mafura, botenya ba ho menahana ha letlalo, le BMI.

Basebetsi ba bongaka ba ngodisitsweng ba tla hula madi ho tswa bakuding pele ho fanwa kalafo ya tlatsetso kapa, nakong eo fanwang ka yona le kamora hoba ho fanwe ka yona, nakong ya selemo se le seng. Disampole tsa madi di tla nkwa ke basebetsi ba bongaka ba ngodisitsweng nakong ya maeto a ditlhatlhobo le maeto a kgwedi le kgwedi, kamorao ho moo ba tla di romela Pathcare (pathology laboratory) mme ke moo di tla hlahlojwang teng ho netefatsa maemo a matla a masole a mmele, maemo a bophelo bo botle, bongata ba kokwanahloko, (kgwedi tse ding le tse ding tse tharo) thuto ya sebopeho sa madi le tshebetso ya sebete (kgwedi tse ding le tse ding tse tharo).

PALO YA BATHO BAO HO ITHUTWANG KA BONA

Baithaopi ba mashome a mahlano ba phelang ka HIV ba tla kgethwa kamora ketelo ya tlhatlhobo, baithaopi ba ileng ba phethahatsa ditlhoko tsa ho kenyelletswa, ba sa phethahatsang ditlhoko tsa ho behellwa ka thoko le ba fanneng ka tumello e ngotsweng fatshe, ba tla kenyelletswa thutong.

Motjha wa ho kenyelletswa

- Banna le basadi ho tloha dilemong tse 18 ho isa ho tse 65 ba nang le HIV/AIDS.
- Palo ya disele tsa $CD4^{+}T$- 200 – 350 cells/mm^3
- Sephetho e be se leng lethathameng le amohelehang mabapi le tsa bongaka, le nalaneng ya ho kula, le tlhatlhobong ya mokudi ka seqo hammoho le ho dumellana le sephetho sa laboratori se ananelwang ke mofuputsi wa bongaka.
- Tjantjello ya ho itlhahisa mabapi le tlhatlhobo ya mmele pele dithuto di qalella le diphuputso tsa laboratori ya pele, le ya ka morao ho dithuto.
- Bokgoni ba ho kutlwisiso le morolo wa ho saena setatemente sa tumello eo o e tsebang ka botlalo.

Motjha wa ho itokolla

Batho ba nale tokelo ya ho itokolla dithutong nakong e nngwe le e nngwe ho sa natswe lebaka, ntle le ho bea tlhokomelo ya bona ya bongaka kotsing. Bakudi bana ba nkwa jwaloka batho ba nyahladitseng morero. Bakudi ba nyahladitseng morero bao e leng hore moriana o lwantshang kokwanahloko oo ho ithuwang ka ona one o sebediswa ho bona, ba tla kopjwa ho kenela tlhatlhobo tsa ha ho se ho qetilwe ka dithuto.

Dintlha tse latelang di ka nna tsa lebisa boitokollong:

6.3.2.1 Ketsahalalo tse seng monate ka baka la ho sebedisa moriana o lwantshang kokwanahloko oo ho ithuwang ka ona, ke ho latela qeto ya mofuputsi wa bongaka.

6.3.2.2 Bakudi ba tlolang metjha e latelwang, ke ho latela qeto ya mofuputsi wa bongaka.

Boitsebiso ba mokudi

- Mokudi e mong le e mong ya ngodisitsweng o tla fumana nomoro (01 – 50) mme o tla dula ka yona ho fihlella dithuto di fihla pheletsong.
- Mokudi e mong le e mong ya ngodisitsweng o tla dula ka ditlhaku tsa pele tsa mabitso a hae tse fumanweng ka hara khopi ya bukana ya hae ya boitsebiso (eleng tlhaku tse tharo).
- Mokudi e mong le e mong ya ngodisitsweng o tsejwa ka letsatsi la hae la tlhaho (nomoro tse tshelela).

Ho nka karolo ke boithaopo, mme ya hanang ho nka karolo a keke a fumantshwa kotlo kapa tahlehelo ya menyetla eo a nang le tokelo hodima yona; mokudi a ka nna a kgaotsa ho tswelapele nako e nngwe le e nngwe ntle le ho fumantshwa kotlo kapa tahlehelo ya menyetla eo a nang le tokelo hodima yona.

Sephiri

Ho tla etswa matsapa a ho boloka tlhahiso leseding e amang motho e le sephiri. Sephiri se kekeng sa tsebahala ho hang se keke sa netefatswa. Tlhahiso leseding e amang motho e ka nna ya phatlalatswa ebang e batlwa ka molao.

Bahlophisi ba ka nna ba hlahloba kapa ba khopa direkoto tsa hao tsa dipatlisiso mabapi le tiiso ya boleng le qaqiso ya lesedi, mme ba ka nna ba kenyelletsa dihlopha tse jwaloka Ethics Committee for Medical Research and the Medicines Control Council

Dithibelo bakuding

Meriana:
Kaofela meriana e tsamaisanang e tla ngodiswa ka hara CRF. Ebang ho ka hlokwa meriana e meng ya tlatsetso nakong ya dithuto mofuputsi wa bongaka o tla tlameha ho tsebiswa. Meriana e tsamaisanang e tlameha ho bontsha ebang e sena mabitso a itseng a kgwebo kapa e nale ona, e bontshe matla a ona, le ka moo kalafo e ka phetwang ka teng le hore e nka nako e kae.

Dijo:
Bakudi ba tshwanetse ho tswelapele ka dijo tsa bona tse tlwaelehileng.

Polokeho ya bakudi
Porojeke e bolokehile haholo. Baithuti ba tla dula ba disitswe tsatsi le leng le leng nakong eo ba nwang meriana ya bona ya tlatsetso e sebetsanang le kokwanahloko. Moriana wa tlatsetso o sebetsanang le kokwanahloko ke liquid multi vitamin. Ketelong e nngwe le e nngwe, bakudi ba tla hlahlojwa mmeleng le ho hlahloba meriana ya bona ke ngaka. Disampole tsa madi di tla hulwa ho bakudi ho tiisa maemo a bona a ho itwanela kgahlano le kokwanahloko le maemo a bona a bophelo, mme kgwedi tse ding le ding tse tharo ho tla hlahlojwa palo ya masole a mmele le tshebetso ya sebete. Basebetsi ba bongaka ba ngodisitsweng ba tla hula madi ho bakudi pele ho phano ya meriana ya tlatsetso e lwantshang kokwanahloko, le nakong eo ho fanwang ka yona, le kamora hoba ho fanwe ka yona nakong e etsang selemo se le seng.

Maemo a ditjhelete mabapi le bakudi
Haho moo bakudi ba amehang teng mabapi le tefo ya ditjhelete. Bakudi ha ba tlameha ho lefella tlhatlhobo ya bona ya bongaka kapa sephetho se seng le se seng sa bongaka. Ba tla fumantshwa phekolo ntle le tefo. Ba tla boela ba fumantshwa meriana ya bona ya tlatletso e lwantshang kokwanahloko mahala. Nakong ya ketelo e nngwe le e nngwe ba tla fumantshwa dijo tsa mahala le dipalangwang tsa mahala ho ya tliliniking. Baithuti ba ke ke ba lefjwa mabapi le karolo eo ba e nkileng tekong tsena.

MERIANA YA TLATSETSO E LWANTSHANG KOKWANAHLOKO E SEBEDISETSWANG HO ITHUTA KA YONA E BITSWANG DI SUPPLEMENTS
Dikateng tsa di supplements

Ka tlase ho ngotswe dikateng tsa multi-vitamin/anti-oxidant supplement tse tla sebediswa nakong ya diteko.

Bakudi ba tla fumantshwa Pelise engwe hangwe ka letsetsi wa supplement (pakeng tsa 07:00 – 09:00). Basebetsi ba South African Red Cross Community Home Based Care Program ka hara setjhaba, ba tla sebetsana le ho fana ka moriana le ho disa taba ya ho nwa supplement, le hore batho ba phethahatsa ditshwanelo tsatsi le leng le le leng. Bakudi ba tshwanetse ho tswelapele ka dijo tsa bona tse tlwaelehileng.

Dintlha mabapi le bafuputsi bao ho ikopangwang
le bona:

Prof E van den Heever-Kriek (Project leader)
Cell nr: 082 770 5356
Mr. E Vermaak (Researcher)
Cell nr: 082 775 6261
Dr. P Basson (Researcher)
083 414 8445

Dintlha mabapi le moo ho ikopangwang le mongodi le modulasetulo wa REC – mabapi le ho tlaleha ditletlebo/mathata
Mohala nr: 051-405 2812

APPENDIX C

SOCIO-DEMOGRAPHIC QUESTIONNAIRE

(All information in this questionnaire is confidential)

Name: _____

Respondent No.: ☐☐☐ 1-3

Interviewer: ☐☐ 4-5

1. Date of Birth: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐ 6-13

2. Date of interview: ☐☐☐☐☐☐☐☐ 14-21

3. Age (if date of birth is unknown): ☐☐ 22-23

Address: .....

.....

Telephone No. ..... (H) ..... (W)

4. How many years have you been living in an urban area (like Mangaung)? _____ ☐☐ 24-25

5. Language: 26

1. Sotho ☐
2. Tswana ☐
3. English ☐
4. Afrikaans ☐
5. Other, specify .....

Number of children (born): _____ ☐☐ 27-28

Number of children (alive): _____ ☐☐ 29-30

6. Do you smoke at all? 31

1. Yes ☐
2. No ☐

If YES, how many cigarettes per day? ☐☐ 32-33

7. Household composition:

How many persons live in the house permanently
(5-7 days per week)? _____ | | | 34-35
Number of children (< 18 yrs): _____ | | | 36-37
Number of adults (≥ 18 yrs): _____ | | | 38-39

8. Marital status of respondent: 40

1. Unmarried
2. Married
3. Divorced
4. Separated
5. Widowed
6. Living together
7. Traditional marriage
8. Other, specify .....

9. What is your highest level of education? 41

1. None
2. Primary School
3. Std 6-8
4. Std 9-10
5. Tertiary Education
6. Don't know

10. Employment status of respondent 42

1. Unemployed
2. Self Employed
3. Full-time wage earner (receive a salary)
4. Other, specify (part-time, peace job etc.)

   _____
5. Don't know

11. Husband/ partner's employment status 43

1. Retired by choice
2. Unemployed
3. Self Employed
4. Full time wage earner (receive a salary)
5. Other, specify (part-time, peace job etc.) _____
6. Not applicable, e.g. dead

12. Who is the head of this household? 44

1. Self
2. Husband
3. Child(ren)

4. Parent
5. Grandparent
6. Friend
7. Other, specify _____

13. Type of dwelling: ☐ 45

1. Brick, Concrete
2. Traditional mud
3. Tin
4. Plank, wood
5. Other, specify_____

14. Number of rooms in house (excluding bathroom, toilet and kitchen, if separate) ☐☐ 46-47

15. Where do you get drinking water most of the time? ☐ 48

1. Own tap
2. Communal tap
3. River, dam
4. Borehole, well
5. Other, specify _____

16. What type of toilet does this household have? ☐ 49

1. Flush
2. Pit
3. Bucket, pot
4. VIP
5. Other, specify _____

17. What fuel is used for cooking most of the time? ☐ 50

1. Electric
2. Gas
3. Paraffin
4. Wood, Coal
5. Sun
6. Open fire

18. Do you use a cast iron pot for cooking? ☐ 51

1. Never
2. ≤ Once a week
3. > Once a week
4. Every day

19. Does the home have a **WORKING**:

Refrigerator and/or freezer ☐ 52

1. Yes
2. No

Stove (gas, coal or electric) or Hot plate ☐ 53

Yes
No

Primus or Paraffin Stove ☐ 54

1. Yes
2. No

Microwave ☐ 55

1. Yes
2. No

Radio and/or Television ☐ 56

1. Yes
2. No

20. How many people contribute to the total income? _____ ☐☐ 57-58

21. Household income per month (including wages, rent, sales of vegs, etc.,

State grants) _____ ☐ 59

1. None
2. R100 - R500
3. R501 - R1000
4. R1001 - R3000
5. R3001 - R5000
6. Over R5000
7. Don't know

22. Is this more or less the income that you had over the past six months? ☐ 60

1. Yes
2. No

23. If NO, is it More or Less? 61

1. More
2. Less

24. How much money is spent on food weekly? ☐☐ 62-63

1. R0 – R50
2. R51 – R100

3. R101 – R150
4. R151 – R200
5. R201 – R250
6. R251 – R300
7. R301 – R350
8. R351 – R400
9. Over R400

❄ ❄ ❄

APPENDIX D

QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

Name: .....

Respondent number: ☐☐☐ 1-3

Interviewer: ..... ☐☐☐ 4-5

Greeting

Please think carefully about the food and drinks you have consumed during the past 6 months. I will now go through a list of foods and drinks with you and I would like you to tell me:

- if you eat these particular foods,
- how the food is prepared,
- how much of the food you eat at a time, and
- how many times a day you eat it and if you do not eat it every day, how many times a week or a month it is eaten.

To help you to describe the amount of a food, I will show you pictures or models of different amounts of the food. Please say which picture or model is the closest to the amount eaten, or if it is smaller, between sizes or bigger than the pictures or models. Amounts can also be reported as cups (c), tablespoons (T) or teaspoons (t).

- **There are no right or wrong answers.**
- **Everything you tell me is confidential.**
- **Is there anything you want to ask now?**
- **Are you willing to go on with the questions?**

Encircle the appropriate answer.

Do you follow any special diet? Yes (1) No (2) ☐ 6

If Yes, please specify (encircle appropriate answer) ☐ 7

1. Diabetic diet
2. Slimming diet
3. Allergies
4. Other (specify)_____

Do you use salt in your food? Yes (1) No (2) Don't know (3) ☐ 8

Are other, flavoured salts e.g. Aromat used in your food? Yes (1) No (2) Don't know (3) ☐ 9

Please specify _____

Do you use beef/ chicken stock in your food? Yes (1) No (2) Don't know (3) ☐ 10

Do you use any dietary supplements? Yes (1) No (2) Don't know (3) ☐ 11

If Yes, please specify the type (name), how often, and how much:

Vitamins: _____ ☐☐☐ 12-14

Minerals: _____ ☐☐☐ 15-17

Protein: _____ ☐☐☐ 18-20

Energy: _____ ☐☐☐ 21-23

Other: _____ ☐☐☐ 24-26

Eating patterns (frequency of eating):

Please indicate which of the following best describes the eating pattern you usually follow (mark only one) ☐ 27

1. More than three meals, with eating between meals
2. Three meals, with eating between meals
3. Three meals, with no eating between meals
4. Two meals, with eating between meals
5. Two meals, with no eating between meals
6. One meal, with eating between meals
7. One meal, with no eating between meals
8. Nibble the whole day, no specific meals
9. Others (please specify):

Do you eat breakfast? ☐ 28

1. Regularly (≥4 times a week)
2. Sometimes (1 – 3 times a week)
3. Never

How often do you eat at the following places away from home?

Family	1) Never	2) >Once /week	3) Weekly	4) Monthly	5) >Once a month	☐ 29
Friends	1) Never	2) >Once /week	3) Weekly	4) Monthly	5) >Once a month	☐ 30
Café	1) Never	2) >Once /week	3) Weekly	4) Monthly	5) >Once a month	☐ 31
Restaurant, Fast food	1) Never	2) >Once /week	3) Weekly	4) Monthly	5) >Once a month	☐ 32
Other, specify _____	1)	2) >Once	3)	4)	5) >Once	☐ 33

	Never	/week	Weekly	Monthly	a month	

Do you drink coffee with your meals? ☐ 34

1. Yes
2. No

If Yes, at which meals?

Breakfast	1) Yes	2) No	☐	35
Lunch	1) Yes	2) No	☐	36
Supper	1) Yes	2) No	☐	37
Snacks	1) Yes	2) No	☐	38

Do you drink tea (except Rooibos) with your meals? ☐ 39

1. Yes
2. No

If Yes, at which meals?

Breakfast	1. Yes	2. No	☐	40
Lunch	1. Yes	2. No	☐	41
Supper	1. Yes	2. No	☐	42
Snacks	1. Yes	2. No	☐	43

With how many meals per day do you eat meat, fish or poultry? ☐ 44

1. One meal
2. Two meals
3. All meals
4. None

Do you eat fresh fruit and/or vegetables with the following meals?

Breakfast	1. Yes	2. No	☐	45
Lunch	1. Yes	2. No	☐	46
Supper	1. Yes	2. No	☐	47
Snacks	1. Yes	2. No	☐	48

❄ ❄ ❄

Summary of food frequency questionnaire

FOOD	CALCULATIONS	CODE – AMOUNT PER DAY (G)								
										(1-8)
										(9-16)
										(17-24)
										(25-32)
										(33-40)
										(41-48)
										(49-56)
										(57-64)
										(65-72)
										(73-80)
										(1-8)
										(9-16)
										(17-24)
										(25-32)
										(33-40)
										(41-48)
										(49-56)
										(57-64)
										(65-72)
										(73-80)
										(1-8)
										(9-16)
										(17-24)
										(25-32)
										(33-40)
										(41-48)
										(49-56)
										(57-64)
										(65-72)
										(73-80)
										(1-8)
										(9-16)

										(17-24)
										(25-32)
										(33-40)
										(41-48)
										(49-56)
										(57-64)
										(65-72)
										(73-80)
										(1-8)
										(9-16)
										(17-24)
										(25-32)
										(33-40)
										(41-48)
										(49-56)
										(57-64)
										(65-72)
										(73-80)

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Maize-meal porridge	Stiff (pap)						**3400**	
Maize-meal porridge	Soft (slap pap)						**3399**	
Maize-meal porridge	Crumbly (phutu)						**3401**	
Sour porridge	Specify ratio Mabella/ Maize						**3399**	
Mabella porridge	Stiff, coarse, fine						**3437**	
Mabella porridge	Soft, coarse, fine						**3437**	
Oats porridge	Brand name:						**3239**	
Breakfast cereals	Puffed Wheat, plain						**3325**	
	Corn Flakes, plain						**3243**	
	Weet Bix						**3244**	
	Puffed Rice, sweet						**3372**	

	Specify types usually eaten _____ _____ Brand names of cereals available at home now: _____							
Milk on porridge or cereal: Circle type usually used	None							
	Whole/fresh						**2718**	
	Sour						**2787**	
	2% fat						**2772**	
	Fat free/skimmed						**2775**	
	Milk blend						**2771**	
	Soy milk						**2737**	
	Condensed (whole,sweet)						**2714**	
	Condensed (skim, sweet)						**2744**	
	Evaporated whole						**2715**	
	Evaporated low fat						**2827**	
	Non-dairy creamer						**2751**	
Is sugar added to porridge or cereal? (Tick box)	None □ White □ Brown □ Syrup □ Honey □ Sweetener (type): _____						**3989** **4005** **3988** **3984**	
Is fat added to porridge or cereal? (Tick box)	None □ Animal fat (butter) □ Hard margarine □ Soft margarine □ Oil □ Peanut Butter □						**3479** **3484** **3496** **3507** **3485**	
Samp/Maize rice **Samp and beans** **Samp and peanuts**	Bought Self ground Specify ratio (1:1) Specify ratio						**3250** **3725** **3402**	
Rice: specify brand names:	White Brown Sorghum rice						**3247** **3315** **3437**	
Stamped wheat							**3249**	
Pastas	Macaroni Spaghetti Spaghetti in tomato sauce Other: _____						**3262** **3262** **3258**	

How many times a week do you eat porridge or breakfast cereal at any time of the day (NOT ONLY BREAKFAST)?_____

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom/ Never		
Bread/Bread rolls	White						**3210**	
Bread slices: thin	Brown						**3211**	
Medium, thick	Whole wheat						**3212**	
Other breads	Specify types e.g. Raisin Maize meal Sweetcorn Rye Other						**3214** **3278** **3379** **3213**	
Pizza (specify toppings) **Hot Dogs(specify sausage)** **Hamburgers (specify meat)**	Cheese, tomato & onion _____ _____ _____ _____ _____						**3353**	
Are any of the following spreads used on bread? **Fat spreads** (Tick box)	Butter □ Butro □ Animal fat (beef tallow) □ Lard □ Hard margarine (brick) □ Soft margarine (light) □ Cooking Fat □						**3479** **3523** **3494** **3495** **3484** **3496** **3516**	
Peanut butter							**3485**	
Sweet spreads	Jam Syrup Honey						**3985** **3988** **3984**	
Marmite/ OXO/ Bovril							**4030** **4029** **4029**	
Fish paste **Meat paste**							**3109** **2917**	
Cheese	Specify types: Cottage low-fat cheese Cream cheese Gouda Cheddar Other: _____ _____						**2760** **2725** **2723** **2722**	
Cheese spreads	Low fat Full fat Specify types						**4310** **2730**	
Atchar							**3117**	
Other spreads: (Specify tipes)	_____ _____ _____							

Dumpling							**3210**	
Vetkoek							**3257**	
Provita **Crackers (refined)** **Crackers (whole wheat)**							**3235** **3331** **3391**	
Rusks *Home-made:*	Bran Buttermilk White Boerebeskuit, white All-bran Raisins Buttermilk, white Buttermilk, whole wheat Other: _____						**3330** **3329** **3364** **3364** **3380** **3380** **3215** **3255**	
Scones							**3237**	
Muffins	Plain Bran						**3408** **3407**	

How many times a day do you eat bread? _____

	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Chicken **Do you eat the chicken with the skin?** **Yes** □ **No** □	Boiled: with skin without skin						**2926** **2963**	
	Fried: in batter/ crumbs Fried, but not coated						**3018** **2925**	
	Roasted/grilled with skin without skin						**2925** **2950**	
Chicken bones stew							**A003**	
Chicken heads, raw							**2999**	
Chicken stew, with veg & skin							**3005**	
Chicken feet, raw							**2997**	
Chicken offal	Giblets						**2998**	
Chicken pie	Commercial Home-made						**2954** **2954**	
Red meat: Beef	Fried/grilled: with fat / without fat						**2908** **2959**	
	Stewed/boiled: with fat / without fat						**3006** **2909**	
	Mince with tomato and onion						**2987**	
Red meat: Mutton	Fried/grilled: with fat / without fat						**2927** **2934**	
	Stewed/boiled: with fat / without fat						**3040** **2916**	
Red meat: Pork	Fried/grilled: with fat / without fat						**2930** **2977**	
	Stewed/boiled: with fat / without fat						**3046** **3045**	
Red meat: Goat	Fried/grilled: with fat / without fat						**4281**	
	Stewed/boiled: plain / with veg						**4281** **4282**	
Offal (Specify type)	Intestines: boiled, nothing added						**3003**	
	"Vetderm" fried						**3003**	
	Stewed with vegetables							

	Liver						**2955**	
	Kidney						**2956**	
	Tripe "pens" trotters, head						**3003**	
	Pluck (lungs, heart, gullet)						**3019**	
Specify vege-tables used in meat stews (only if not mentioned elsewhere)								
Wors / sausage	Fried						**2931**	
Bacon							**2906**	
Cold meats	Polony						**2919**	
	Ham						**2967**	
	Vienna's canned						**2936**	
	Russian						**2948**	
	Frankfutter						**2937**	
	Other (specify) _____							
Canned meat	Bully beef						**2940**	
	Other (specify)							
Meat pie	Bought						**2939**	

Legumes: specify dried beans / peas / lentils	Stews & curries (specify) Soups Salad						**3157** **3174**	
Baked beans							**3176**	
Soya products e.g. Toppers / Imana	Brands at home now: Don't know _____ (Show examples)						**3196**	
Fried fish (fresh or frozen / fried in sun oil)	With batter/ crumbs Without batter/ crumbs						**3072** **3060**	
Fresh water fish (Specify type)	Specify cooking method: Medium fat, batter, fried						**3094**	
Canned fish: Pilchards	In brine						**3055**	
	In tomato sauce						**3102**	
	Mashed with fried onion						**A005**	

Sardines	In oil In tomato sauce						**3087** **3087**	
Tuna	In oil In brine						**3093** **3054**	
Mackerel							**3113**	
Salmon							**3101**	
Pickled fish/ curried							**3076**	
Do you remove fish bones before eating canned fish	YES ☐ NO ☐							
Fish cakes Specify canned or other	Fried: oil / butter / margarine, commercial						**3080**	
Salted dried fish							**3077**	
Eggs	Boiled/poached Scrambled in: oil butter margarine Fried in: oil butter margarine bacon fat Curried						**2867** **2889** **2886** **2887** **2869** **2868** **2877** **2870** **2902**	

How many times a week do you eat:

Meat .....

Beans .....

Chicken .....

Fish .....

Eggs .....

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT /DAY
			Per day	Per week	Per month	Seldom / Never		
Cabbage	Boiled, nothing added						**3756**	
	Boiled with potato, onion and fat						**3813**	
	Fried, in margarine (nothing added)						**3810**	
	Fried, in oil (nothing added)						**3912**	
	Boiled, then fried with potato, onion						**A006**	
	Other: _____							
Spinach / morogo /	Boiled, nothing added						**3913**	

imfino / other green leafy vegetables: List names	Boiled, fat added (margarine)						**3898**	
	Boiled with onion / tomato and fat						**A011**	
	- onion & potato (margarine)						**3901**	
	- onion, tomato & potato							
	- with peanuts							
	Other: _____							
Tomato and onion 'gravy' / relish / chow	Home made: - with fat - without fat						**3910** **3925**	
	Canned						**4129**	

Pumpkin (Specify type)	Cooked in fat and sugar						**A010**	
	Boiled, little sugar and fat						**A010**	
	Boiled						**4164**	
	Other: _____							
Carrots	Boiled, sugar & fat						**3819**	
	Boiled, nothing added						**3757**	
	Boiled, potato, onion, no fat						**3934**	
	Boiled, potato, onion, margarine						**3822**	
	Boiled, with sugar						**3818**	
	With potato/onion						**3934**	
	Raw, salad (orange juice)						**3711**	
	Chakalaka							
	Other: _____							
Mealies / Sweet corn	On cob						**3725**	
	Off cob - creamed sweet corn Off cob - whole kernel						**3726** **3942**	
Beetroot	Cooked, Salad (bought or home-made)						**3698** **3699**	

Potatoes	Boiled - with skin						**4155**	
	- without skin						**3737**	

	Baked in skin (flesh and skin) - in skin (flesh only)						**3736 3970**	
	Mashed - skim milk, margarine						**3875**	
	Mashed - whole milk, margarine						**3876**	
	Roasted in beef fat						**3878**	
	French fries / potato chips (oil)						**3740**	
	Salad (mayon-naise and egg)						**3928**	
	Other: _____							
Sweet potatoes	Boiled with skin						**3748**	
	- without skin						**3903**	
	Baked with skin						**3748**	
	- without skin						**3903**	
	Mashed						**3903**	
	Other: _____							

Peas	Green, frozen						**4146**	
	Green, frozen with sugar						**3720**	
	With sugar and butter						**3859**	
	Tinned peas						**4149**	
Green peppers	Raw Cooked (stew with oil)						**3733 3865**	
Brinjal / egg plant	Cooked Fried in oil Stew (oil, onions, tomato)						**3700 3802 3798**	
Mushrooms	Raw Sautéed in brick margarine Sautéed in oil						**3842 3839 3841**	
Onions	Sautéed in sun oil Sautéed in marga-rine						**3730 3844**	
Salad vegetables	Raw tomato						**3750**	
	Lettuce						**3723**	
	Cucumber						**3718**	
	Avocado's						**3656**	
Green Beans	Boiled, nothing added						**3696**	
	Cooked, potato, onion, margarine						**3792**	

	Cooked, potato, onion, no fat						**3933**	
Cauliflower	Boiled						**3716**	
Other vegetables (Specify)	_____ _____							
If you fry veg or add fat, specify type of fat usually used	Butter ☐ Butro ☐ Animal fat (beef tallow) ☐ Lard ☐ Hard margarine (brick) ☐ Soft margarine (tub) ☐ Soft margarine (light) ☐ Sunflower oil ☐						**3479** **3523** **3494** **3495** **3484** **3496** **3524** **3507**	

How many times a week do you eat vegetables? _____

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT /DAY
			Per day	Per week	Per month	Seldom /Never		
Mayonnaise / salad dressing	Mayonnaise: - bought - home-made Cooked salad dressing Salad dressing low-oil Salad dressing French Oil: - Olive - Sunflower - Canola						**3488** **3506** **3503** **3505** **3487** **3509** **3507** **4280**	
Apples	Fresh Canned, unsweet-ened						**3532** **4216**	
Pears	Fresh Canned, in syrup						**3582** **3583**	
Bananas							**3540**	
Oranges **Naartjie**							**3560** **3558**	
Grapes							**3550**	
Peaches	Fresh Canned in syrup						**3565** **3567**	
Apricots	Fresh Canned in syrup						**3534** **3535**	
Mangoes	Fresh						**3556**	
Pawpaw	Raw						**3563**	
Pineapple	Raw Canned (syrup)						**3581** **3648**	
Guavas	Fresh Canned (syrup)						**3551** **3553**	
Watermelon							**3576**	
Spanspek	Orange flesh Green flesh						**3541** **3575**	
Wild fruit / berries (Specify types)	_____ _____ _____ _____ _____							

Dried fruit (also as snacks)	Raisins Prunes (raw) Prunes (cooked with sugar) Peaches (raw) Peaches (cooked with sugar) Apples (raw) Dried fruit sweets Other: _____ _____ _____ _____						**3552** **3596** **3564** **3568** **3569** **3600** **3995**	
Other fruit	_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____		_____ _____ _____ _____

How many times a week do you eat fruits? _____

WE NOW WILL ASK YOU QUESTIONS ABOUT WHAT YOU USUALLY DRINK

BEVERAGES	DESCRIPTION	AMOUNT USUALLY TAKEN	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Water							**4042**	
Tea	Ceylon Rooibos						**4038** **4054**	
Coffee							**4037**	
Sugar per cup of tea or coffee	White Brown						**3989** **4005**	
Milk per cup of tea or coffee What type of milk do you put in tea and/or coffee?	Fresh / Long life / Whole						**2718**	
	Fresh / long life 2%						**2772**	
	Goat						**2738**	
	Fresh / Long life / fat free (skimmed)						**2775**	
	Whole milk powder, reconstituted Specify brand: _____						**2831**	
	Skimmed milk powder, reconstituted Specify brand: _____						**2719**	
	Milk blend, reconstituted Specify brand: _____						**2771**	

	Whitener / non-dairy creamer Specify brand: _____						**2751**	
	Condensed milk (whole)						**2714**	
	Condensed milk (skimmed)						**2744**	
	Evaporated milk (whole)						**2715**	
	Evaporated milk (low-fat)						**2827**	
	None							
Milk as such: What type of milk do you drink?	Fresh / long life / whole						**2718**	
	Fresh/longlife/fat free (skimmed)						**2772**	
	Fresh/long life/2%						**2775**	
	Goat						**2738**	
	Sour / Maas						**2787**	
	Buttermilk						**2713**	

BEVERAGES	**DESCRIPTION**	**AMOUNT USUALLY TAKEN**	**TIMES TAKEN**				**CODE**	**AMOUNT / DAY**
			Per day	**Per week**	**Per month**	**Seldom / Never**		
Milk drinks Specify brands, Including milk supplements and type of milk used	Nestle Nesquik _____ Milo _____ Flavoured milk _____ Other _____						**4287** **2735** **2774**	
Yoghurt	Drinking yoghurt Thick yoghurt, plain, fruit						**2756** **2732**	
Squash	SixO Oros Lecol with sugar -artificial sweetener Kool Aid Other _____ _____ _____						**3990** **3982** **3982** **3990** **3982**	
Fruit juice	Fresh / Liquifruit / Ceres						**2866**	
	"Tropica"/ mixtures with milk						**2791**	
Fruit syrups	Average Guava syrup						**2865** **2864**	
Fizzy drinks (Coke, Fanta)	Sweetened Diet						**3981** **3990**	

Mageu/Motogo							**4056**	
Alcoholic beverages such as Sorghum beer	Sorghum beer Specify: _____						**4039**	
Other (specify)	Beer average Wine Cider _____ _____ _____ _____ _____ _____						**4031** **4033** **4057**	

PLEASE INDICATE WHAT TYPES AND AMOUNTS OF SNACKS, PUDDINGS AND SWEETS YOU EAT:

FOODS	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Potato crisps / chips							**3417**	
Peanuts	Roasted, unsalted Roasted, salted						**3452** **3458**	
Cheese curls: Niknaks etc.	Average Savoury						**3267** **3418**	
Popcorn	Plain (no salt and butter) Plain (salt and butter added) Sugar coated						**3332** **3359**	
Raisins (seeds)							**4231**	
Chocolates	Milk Kit Kat Peppermint crisp Specify types and names _____ _____						**3987** **4024** **3997**	
Candies	Sugus, gums, hard sweets (specify) _____ Peppermint						**3986** **4004**	
Sweets	Toffees Hard boiled Fudge, caramels (specify) _____						**3991** **3986** **3991**	
Biscuits / cookies	Specify type Home made plain Shortbread, butter Commercial, plain Commercial with filling						 **3233** **3296** **3216** **3217**	
Cakes and tarts	Chocolate, plain						**3419**	
Pancakes/ crumpets							**3344**	
Koeksisters							**3231**	

Savouries	Sausage rolls Samoosa – vegetable Samoosa – mutton Biscuits e.g. Bacon kips Other: _____ _____						**2939** **3414** **3355** **3331**	
Pudding: jelly							**3983**	
Baked pudding	Plain batter						**3429**	
Instant pudding	Skim milk Whole milk						**3314** **3266**	
Ice cream	Commercial regular Commercial rich Soft serve Sorbet Ice lollies Chocolate coated individual ice creams (e.g. Magnum)						**3483** **3519** **3518** **3491** **3982**	
Custard	Home made, whole milk Ultramel						**2716** **2716**	
Cream	Fresh						**3520 / 3480**	
Other pud-dings (Specify):	_____ _____ _____							

HOW MANY TIMES A WEEK DO YOU EAT SNACK FOODS? _____

SAUCES / GRAVIES / CONDIMENTS

FOODS	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Tomato Sauce / Worcester sauce							**3139** **4309**	
Chutney	Fruit						**3168**	
	Tomato						**3114**	
Pickles							**3866**	
Packet soups							**3158**	
Beef / chicken stock							**4029**	
Others:	_____ _____ _____							

Wild birds, animals, insects or fruits and berries (hunted or collected in rural areas or on farms (specify):

- **Please mention any other foods you eat more than once every two weeks which we have not talked about and/or foods eaten in other homes or places during the past week:**

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		

Are there any foods that you eat which we haven't talked about? Please list them:

FOODS	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		

Thank you for your co-operation and patience.
Good bye!

ADAPTED FROM THE QUESTIONNAIRES OF THE THUSA STUDY (WITH ACKNOWLEDGEMENT TO THE RESEARCH GROUP OF PUCHO) AND THE NATIONAL FOOD CONSUMPTION SURVEY

APPENDIX E

ANTHROPOMETRY INFORMATION

Name: _____

Respondent number: [ ][ ][ ] 1-3

Measurer (interviewer):_____ [ ][ ] 4-5

Weight (kg):_____ [ ][ ][ ].[ ] 6-10

Height (m): _____ [ ].[ ][ ] 11-14

Circumferences (cm):

Upper-arm:_____ [ ][ ].[ ] 15-18

Waist: _____ [ ][ ][ ].[ ] 19-23

Hip: _____ [ ][ ][ ].[ ] 24-28

Age (yrs):_____ [ ][ ] 29-30

Elbow width (cm): _____ [ ].[ ] 31-33

Bodystat count: _____ [ ][ ][ ] 34-36

Frame size [ ] 37

1. Small
2. Medium
3. Large

% Fat: _____ [ ][ ].[ ] 38-41

*% Lean mass:*_____ [ ][ ].[ ] 42-45

APPENDIX F

ANTHRPOLOGY RESULTS

Graphs for descriptive statistics over time

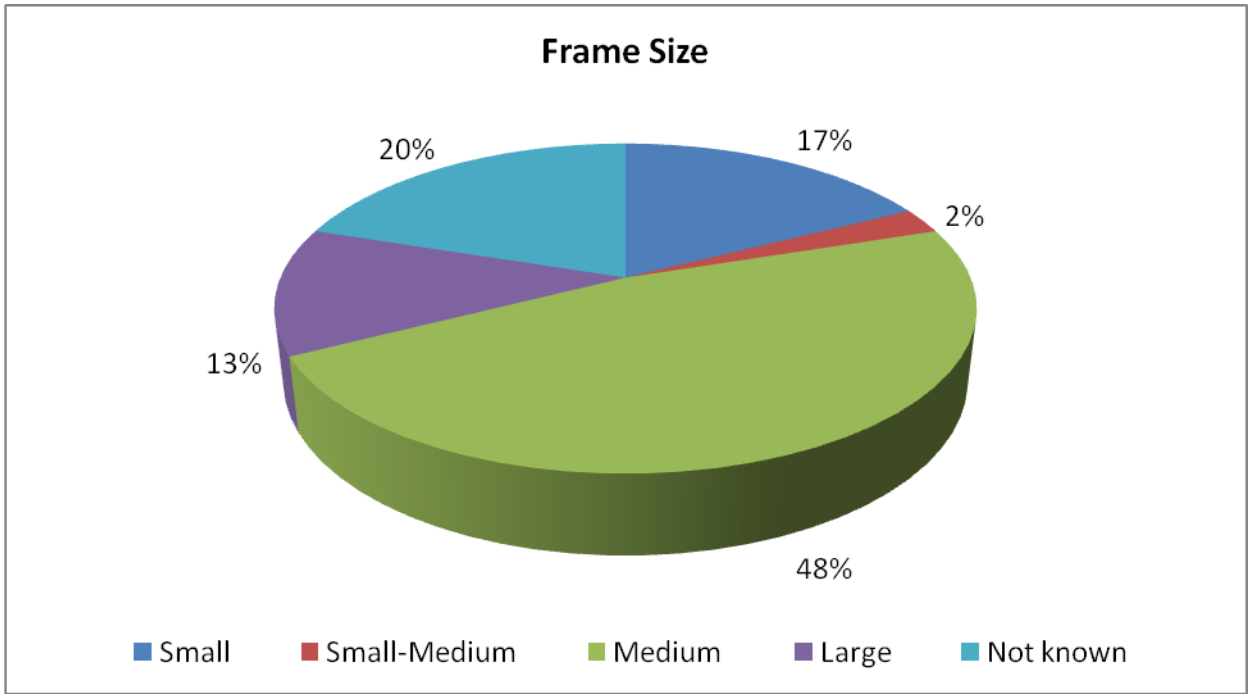


Figure 1: Frame size of infected individuals.

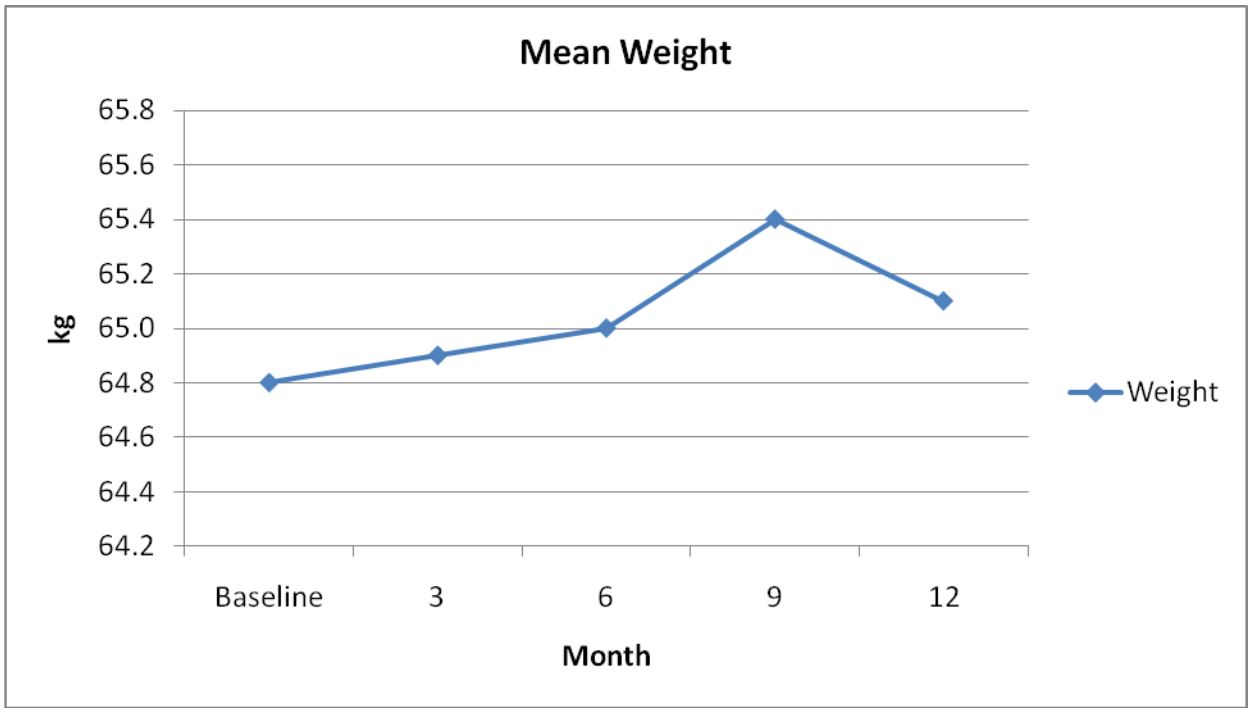


Figure 2: Mean weight over time.

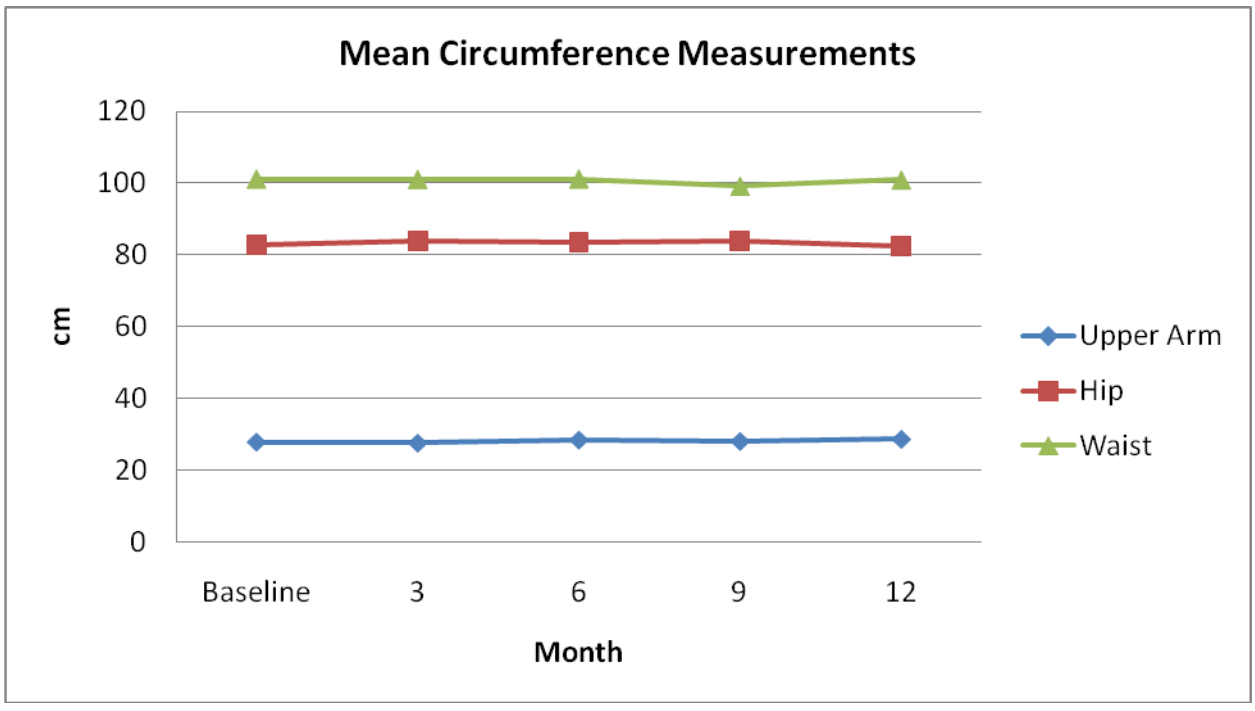


Figure 3: Mean circumference measurements of infected individuals over time.

Figure 4: Mean elbow width of infected individuals over time.

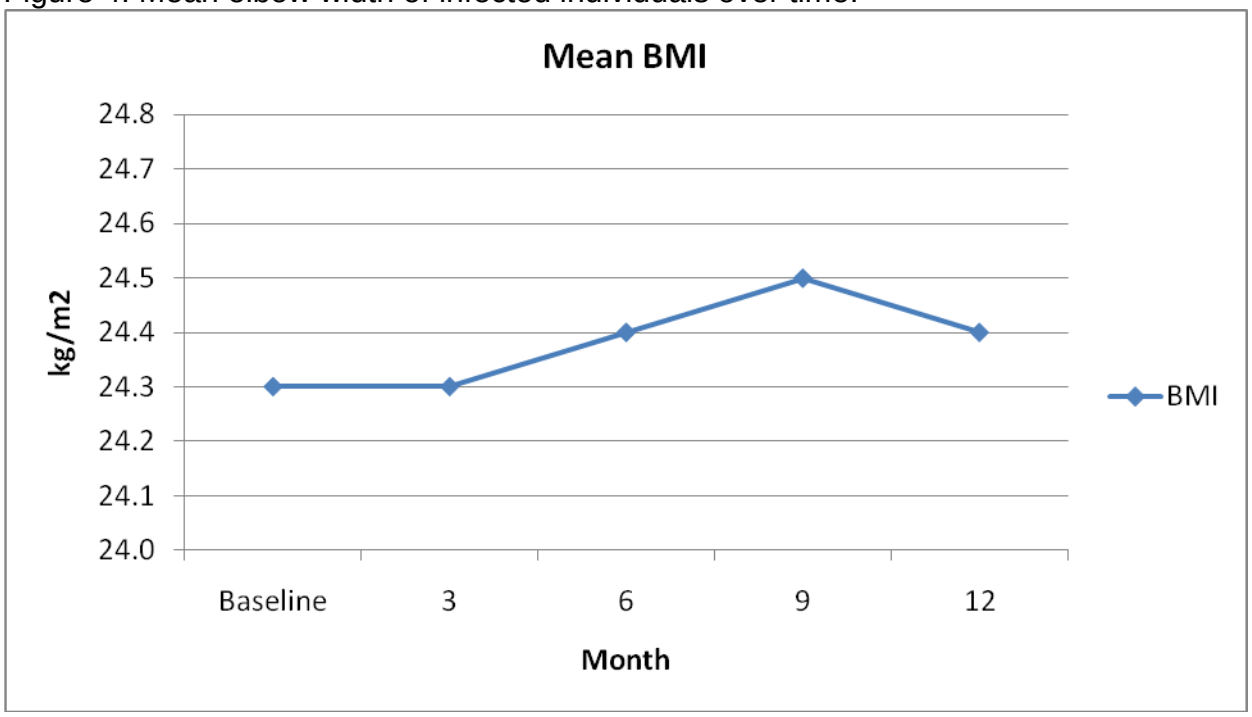


Figure 5: Mean BMI infected individuals over time.

Food Frequencies – Descriptive statistics

The FREQ Procedure

Frame_Size				
Frame_Size	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
Large	**5**	**12.50**	**5**	**12.50**
Medium	**19**	**47.50**	**24**	**60.00**
Not known	**8**	**20.00**	**32**	**80.00**
Small	**7**	**17.50**	**39**	**97.50**
Small-Medium	**1**	**2.50**	**40**	**100.00**

Antropometrie - Descriptive statistics

The MEANS Procedure

Variable	N	Mean	Std Dev	Median	Lower Quartile	Upper Quartile	Minimum	Maximum
Age	40	39.9	9.1	38.5	33.5	46.0	26.0	64.0
Height	40	1.6	0.1	1.6	1.6	1.7	1.5	1.8
Weight_Baseline	40	64.8	10.8	64.6	56.6	71.9	42.0	91.1
Weight_Date3	40	64.9	11.3	65.4	56.8	72.4	40.6	90.6
Weight_Date6	40	65.0	11.0	65.8	56.8	72.4	41.0	91.1
Weight_Date9	40	65.4	11.5	66.4	56.8	71.4	41.8	92.0
Weight_Date12	40	65.1	11.4	66.2	56.7	71.4	42.3	92.3
Circ_Upper_Arm_Baseline	39	27.8	2.8	28.0	26.0	29.0	22.0	37.0
	40	27.5	2.7	28.0	26.0	29.0	21.0	35.0
Circ_Upper_Arm_Date3	40	28.4	2.6	28.7	26.8	29.6	22.0	35.4
	40	28.0	3.2	28.0	26.3	29.4	22.0	34.5
Circ_Upper_Arm_Date6	40	28.6	4.8	28.0	25.8	30.0	22.0	51.0
	40	82.8	9.1	81.0	76.0	90.0	70.0	112.0
Circ_Upper_Arm_Date9	40	83.8	9.5	82.5	77.8	90.0	67.0	111.0

Variable	N	Mean	Std Dev	Median	Lower Quartile	Upper Quartile	Minimum	Maximum
Circ_Upper_Arm_Date12	40	83.4	10.0	82.5	76.0	90.6	63.0	111.5
Circ_Waist_Baseline	40	83.9	10.5	84.6	75.5	91.3	63.0	105.2
Circ_Waist_Date3	40	82.5	9.8	81.9	75.5	87.1	63.0	109.2
Circ_Waist_Date6	40	101.2	9.5	100.5	93.3	107.5	86.0	124.0
Circ_Waist_Date9	40	101.1	10.1	102.5	91.5	109.0	81.0	121.0
Circ_Waist_Date12	40	101.2	10.0	102.0	92.4	109.4	84.0	124.5
Circ_Hip_Baseline	40	99.1	17.4	100.3	92.0	108.3	10.3	126.0
Circ_Hip_Date3	40	100.9	9.6	100.1	94.5	107.2	83.0	124.3
Circ_Hip_Date6	40	5.5	1.7	5.3	4.0	7.0	3.5	9.0
Circ_Hip_Date9	38	4.6	1.5	4.0	3.5	5.0	3.0	8.2
Circ_Hip_Date12	39	4.7	1.4	4.0	4.0	5.0	3.0	8.0

Antropometrie - Differences (Baselins vs. Date6 vs. Date12)

The MEANS Procedure

Variable	N	Median	Lower Quartile	Upper Quartile	Mean	Std Dev	Minimum	Maximum	*p-value (Signed Rank Test – Significant median difference)*
Diff_B_6_Weight	34	-0.55	-2.00	1.00	-0.20	2.38	-4.30	5.00	*0.4943*
Diff_B_12_Weight	34	-0.15	-2.40	1.50	-0.34	3.50	-9.00	8.20	*0.6084*
Diff_6_12_Weight	34	0.10	-1.30	1.50	-0.15	2.84	-7.60	6.00	*0.9096*
Diff_B_6_Circ_Upper_Arm	33	-1.00	-1.50	0.00	-0.60	1.59	-3.20	3.00	*0.0450 **
Diff_B_12_Circ_Upper_Arm	33	-0.80	-1.80	1.00	-0.28	2.18	-3.80	6.00	*0.3015*
Diff_6_12_Circ_Upper_Arm	34	0.00	-0.60	1.00	0.33	1.83	-3.00	5.00	*0.6121*
Diff_B_6_Circ_Waist	34	-0.60	-3.50	3.00	-0.20	5.21	-13.00	10.50	*0.7250*
Diff_B_12_Circ_Waist	34	0.00	-3.70	2.80	0.12	4.64	-8.00	11.70	*0.9646*
Diff_6_12_Circ_Waist	34	0.25	-2.00	2.30	0.32	3.41	-6.20	7.30	*0.6055*
Diff_B_6_Circ_Hip	34	0.00	-1.50	3.00	-0.05	4.97	-11.50	13.50	*0.8409*
Diff_B_12_Circ_Hip	34	0.15	-2.20	4.00	0.12	5.06	-13.00	11.50	*0.5634*
Diff_6_12_Circ_Hip	34	0.40	-1.50	2.00	0.18	3.90	-11.00	9.50	*0.5828*
Diff_B_6_BMI	34	-0.20	-0.70	0.40	-0.07	0.92	-1.60	2.10	*0.4831*
Diff_B_12_BMI	34	0.00	-0.90	0.50	-0.14	1.38	-4.00	3.00	*0.6805*
Diff_6_12_BMI	34	0.00	-0.40	0.60	-0.07	1.13	-3.40	2.50	*0.8417*

APPENDIX G

LIVER FUNCTION TESTING

UNICEL DXC 880i
SYNCHRON ACCESS CLINICAL SYSTEM

METHODOLOGY:

ALB

ALB reagent is used to measure albumin concentration by a timed endpoint method.1,2 In the reaction, albumin combines
with bromcresol purple (BCP) to form a colored product.
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette.
The ratio used is one part sample to 100 parts reagent. The System monitors the change in absorbance at 600
nanometers. This change in absorbance is directly proportional to the concentration of ALB in the sample and is used
by the System to calculate and express ALB concentration.

ALP

ALP reagent is used to measure alkaline phosphatase activity by a kinetic rate method using a
2-amino-2-methyl-1-propanol (AMP) buffer.1,2,3,4,5,6 In the reaction, alkaline phosphatase catalyzes the hydrolysis of
the colorless organic phosphate ester substrate, p-nitrophenylphosphate, to the yellow colored product, p-nitrophenol,
and phosphate. This reaction occurs at an alkaline pH of 10.3.
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette.
The ratio used is one part sample to 50 parts reagent. The system monitors the change in absorbance at 410 nanometers.
This change in absorbance is directly proportional to the activity of ALP in the sample and is used by the System to
calculate and express ALP activity.

ALT

ALT reagent is used to measure analyte activity by a kinetic rate method.1,2 In the reaction, alanine aminotransferase
catalyzes the reversible transamination of L-alanine and alpha-ketoglutarate to pyruvate and L-glutamate. The pyruvate
is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of reduced
â-nicotinamide adenine dinucleotide (NADH) to â-nicotinamide adenine dinucleotide (NAD).
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette.
The ratio used is one part sample to 11 parts reagent. The system monitors the change in absorbance at 340 nanometers.

This change in absorbance is directly proportional to the activity of ALT in the sample and is used by the System to
calculate and express the ALT activity

AST

The AST reagent is used to measure aspartate aminotransferase activity by an enzymatic rate method.1,2 In the
assay reaction, the AST catalyzes the reversible transamination of L-aspartate and á-ketoglutarate to oxaloacetate
and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase (MDH) with
the concurrent oxidation of â-Nicotinamide Adenine Dinucleotide (reduced form) (NADH) to â-Nicotinamide Adenine
Dinucleotide (NAD).
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette.
The ratio used is one part sample to 11 parts reagent. The system monitors the rate of change in absorbance at 340
nanometers over a fixed-time interval. This rate of change in absorbance is directly proportional to the activity of AST in
the sample and is used by the SYNCHRON® System(s) to calculate and express the AST activity.

GGT

GGT reagent is used to measure the ã-glutamyl transferase activity by an enzymatic rate method.1 In the
reaction, the ã-glutamyl transferase catalyzes the transfer of a gamma-glutamyl group from the colorless substrate,
ã-glutamyl-p-nitroaniline, to the acceptor, glycylglycine with production of the colored product, p-nitroaniline.
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette.
The ratio used is one part sample to 20 parts reagent. The system monitors the change in absorbance at 410 nanometers.
This change in absorbance is directly proportional to the activity of GGT in the sample and is used by the System to
calculate and express GGT activity

TBIL

TBIL reagent is used to measure the total bilirubin concentration by a timed endpoint Diazo method.1,2,3 In the reaction,
the bilirubin reacts with diazo reagent in the presence of caffeine, benzoate, and acetate as accelerators to form
azobilirubin.
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The
ratio used is one part sample to 35 parts reagent. The system monitors the change in absorbance at 520 nanometers.

This change in absorbance is directly proportional to the concentration of TBIL in the sample and is used by the System
to calculate and express TBIL concentration.

DBIL
DBIL reagent is used to measure DBIL concentration by a timed endpoint diazo method.1,2 In the reaction, DBIL combines
with diazo to form azobilirubin.
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette.
The ratio used is one part sample to 32 parts reagent. The system monitors the change in absorbance at 560 nanometers.
This change in absorbance is directly proportional to the concentration of DBIL in the sample and is used by the System
to calculate and express the DBIL concentration.
TP
TP reagent is used to measure the total protein concentration by a timed-endpoint biuret method.1 In the reaction, the
peptide bonds in the protein sample bind to cupric ions in an alkaline medium to form colored peptide/copper complexes.
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The
ratio used is one part sample to 50 parts reagent. The System monitors the change in absorbance at 560 nanometers.
This change in absorbance is directly proportional to the concentration of TP in the sample and is used by the System
to calculate and express the TP concentration.
LD
catalyzes the reversible oxidation of L-lactate to pyruvate with the concurrent reduction of â-nicotinamide adenine
dinucleotide (NAD) to reduced â-nicotinamide adenine dinucleotide (NADH).
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The
ratio used is one part sample to 20 parts reagent. The system monitors the change in absorbance at 340 nanometers.
This change in absorbance is directly proportional to the activity of lactate dehydrogenase in the sample and is used by
the System to calculate and express the lactate dehydrogenase activity.

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