THE INFLUENCE OF A MULTIPLE COMBINATION LIQUID PRODUCT ON THE IMMUNE STATUS OF HIV-POSITIVE/AIDS PATIENTS

Thesis submitted by

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BLOEMFONTEIN
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DECLARATION

I, **Oluwafemi Omoniyi Oguntibeju**, student number [redacted] do hereby declare that the research work submitted to the Central University of Technology, Free State for the degree: **Doctor Technologiae: Biomedical Technology** is an original and independent work.

This work has not been submitted to any institution by myself or, to the best of my knowledge, any other person in fulfilment of requirements for the attainment of any qualification.

....
Oluwafemi O. Oguntibeju

14-02-2005
....
Date

DEDICATION

This thesis is dedicated to my wife, Faustina Oguntibeju and my son, Olujare Oguntibeju. They are beautiful gifts from a faithful and loving God.

SUMMARY

The relationship between nutrition and HIV infection/AIDS is well recognised. HIV infection compromises the nutritional status of infected persons and in turn, poor nutritional status affects the progression of HIV infection.

Nutritional supplementation has been shown to be associated with a significant slowing of disease progression, assists in maintaining and optimising the nutritional status and the immune function of HIV-infected persons. The value of nutritional supplementation on the immune function of HIV-positive/AIDS patients from low socio-economic communities in the Free State is not yet known. Hence, the main purpose of this study was to determine the influence of a nutritional supplement (Africa's Solution) on the immune status of HIV-positive/AIDS patients from the low socio-economic communities of Bloemfontein in the Free State Province.

The study entailed a clinical trial that consisted of one screening visit and three monthly visits. A total of 35 respondents were selected according to specific inclusion criteria. Food frequency questionnaires were completed during the screening visit. Haematological parameters, $CD4^{+}$T-cell counts and $CD8^{+}$T-cell counts were determined at the screening visit, monthly visits and by the end of nutrient supplementation (final visit).

The patients studied, demonstrated energy and dietary intake of major macronutrients, higher than the RDA/AI and higher ($P<0.05$) in the male than in the female subjects. It was also observed that the mineral and trace element intakes exceeded the RDA/AI, except for iodine and selenium. A majority of the patients reported adequate intake of most vitamins with the exception of folate and vitamin D. It is envisaged that the high dietary intake of major macronutrients and micronutrients would help in maintaining the nutritional status and in curtailing wasting in the patients.

The anthropometric profiles and the viral load were determined at baseline (n= 35) and at the end of study (n=28). There was no significant difference ($P>0.05$) in the fat percentage and body weight before and after nutrient supplementation; however, fat percentage differed significantly ($P<0.05$) between genders. The body mass index (BMI) and the lean body mass (LBM) produced a trend towards an improvement. There was a positive

correlation between BMI and fat percentage. The CD4$^+$T-cell count showed no correlation with the anthropometric profiles, while the viral load showed a negative correlation with the LBM, the fat percentage and the BMI.

Results of the influence of the supplement on the immune status, haematological and clinical conditions showed that the viral load decreased significantly ($P<0.002$) with time following supplementation. The mean cell volume (MCV) and the mean cell haemoglobin concentration (MCHC) increased significantly ($P<0.002$, $P<0.0002$ respectively), reflecting the positive effect of the supplement on a few of the haematological parameters. The supplement demonstrated no effect on the CD4$^+$T-cell count and the CD4$^+$T-cell count decreased significantly ($P<0.05$) with HIV disease progression. The non-positive effect of the supplement on the CD4$^+$T-cell count may be related to the already low CD4$^+$T-cell counts before supplementation (lower than 200 cells/mm^3 in the majority of patients); short duration; inter-assay variation; changes due to inter-current illness; impaired production of CD4$^+$T-cells; redistribution within the intravascular spaces and drug-nutrient interactions. The supplement showed observable positive effects on the general well-being (clinical conditions) of the patients.

Although, the nutritional supplement did not indicate a positive effect on the CD4$^+$T-cell counts, the reduction in the viral load is very important, since median survival time is known to increase with reduction in HIV viral load. Because of certain limitations (small sample size, short duration, late stage of the infection and inter-assay variation), further study is needed to confirm the efficacy of the supplement.

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OPSOMMING

Die verwantskap tussen voeding en MIV infeksie/VIGS is welbekend. MIV-infeksie kompromiteer die voedingstatus van geïnfekteerde persone, en op sy beurt, beïnvloed swak voedingstatus die progressiewe toename van MIV-infeksie.

Dit is reeds bewys dat die inneem van 'n voedingsupplement die gang van die siekte vertraag, en daartoe bydra om die voedingstatus en die immuunfunksie van MIV-besmette persone te handhaaf en optimaliseer. Die waarde van voedingsupplementasie teenoor die immuunfunksie van MIV-positiewe/VIGS pasiënte, vanuit die lae sosio-ekonomiese gemeenskappe in die Vrystaat, is nog onbekend. Derhalwe was die hoofdoel van hierdie studie om die invloed van voedingsupplementasie op die immuunstatus van MIV-positiewe/VIGS pasiënte, vanuit die lae sosio-ekonomiese gemeenskappe van Bloemfontein, in die Vrystaat, te bepaal.

Die studie het 'n kliniese toets behels wat bestaan het uit een siftingsbesoek en drie maandelikse besoeke. 'n Totaal van 35 respondente is gekies volgens bepaalde insluitingskriteria. Voedselfrekwensievraelyste is voltooi tydens die siftingsbesoek. Hematologiese parameters, $CD4^{+}$T-seltellings en $CD8^{+}$T-seltellings is gedoen tydens die siftingsbesoek, die maandelikse besoeke en aan die einde van die toediening van die voedingsupplement (laaste besoek).

Die pasiënte wat bestudeer is het energie en dieetinames van die belangrikste makrovoedingstowwe getoon wat hoër was as die ADT/AI en die innames was ook hoër ($P<0.05$) in manlike as in vroulike pasiënte. Dit is ook waargeneem dat die minerale- en spoorelementinname die ADT/AI oorskry het, behalwe wat betref jodium en selenium. 'n Meerderheid pasiënte het voldoende innames van die meeste vitamienes gerapporteer, met uitsondering van folaat en vitamien D. Dit word in die vooruitsig gestel dat die hoë inname van makro- en mikrovoedinstowwe sal bydrae om die voedingstatus van pasiënte te handhaaf en om uittering te beperk.

Die antropometriese profiele en die viruslading van die pasiënte is aan die begin van die studie (n=35) en aan die einde van die studie (n=28) bepaal. Daar was geen statisties beduidende verskil ($P>0.05$) in die vetpersentasie en liggaamsmassa voor en na die toediening van die voedingsupplement nie; alhoewel die vetpersentasie aansienlik verskil

($p<0.05$) het tussen geslagte. Die l[illegible]ndeks, die middllyf-heup-verhouding en die maerligaamsmassa het 'n neiging tot verbetering getoon. Daar was 'n positiewe korrelasie tussen liggaamsmassa-indeks en vetpersentasies. Die $CD4^+$T-seltelling het geen korrelasie met die antropometriese profiele getoon nie, terwyl die viruslading 'n negatiewe korrelasie met die maerliggaamsmassa, die vetpersentasie en die liggaamsmassa-indeks getoon het, maar wel 'n positiewe korrelasie met die middellyf-heup verhouding.

Resultate van die invloed van die voedingsupplement op die immuunstatus, die hematologiese en kliniese toestande, het getoon dat die viruslading aansienlik gedaal het ($P<0.002$) met verloop van tyd na die toediening van die supplement. Die gemiddelde korpuskulêre volume en die gemiddelde korpuskulêre hemoglobienkonsentrasie het aansienlik vermeerder ($P< 0.002$, $P<0.0002$ respektiewelik), wat die positiewe effek van die voedingsupplement op sommige van die hemoglobienparameters aantoon. Die voedingsupplement het geen effek op die $CD4^+$ T-seltelling gehad nie en die $CD4^+$T-seltelling het aansienlik verminder ($P<0.05$) soos wat die MIV-siekte toegeneem het. Die nie-positiewe effek van die voedingsupplement op die $CD4^+$ T-seltelling kan moontlik verband hou met die reeds lae $CD4^+$ T-seltellings vòòr die toediening van die supplement (laer as 200 selle/mm^3 in die meeste pasiënte), kort tydsduur; inter-toets variasie; verandering as gevolg van bykomende siektetoestande; verswakte selproduksie; herverdeling binne die intravaskulêre ruimtes en medikasie-voedingstof-interaksie. Die voedingsupplement het waarneembare positiewe gevolge getoon op die algemene welstand (kliniese toestand) van die pasiënte.

Alhoewel die voedingsupplement nie 'n positiewe effek op die $CD4^+$ T-seltelling gehad het nie, was die afname van die viruslading baie belangrik, omdat dit bekend is dat die mediaan-oorleweringstyd vermeerder soos wat die viruslading verminder. As gevolg van sekere beperkings (klein steekproefgrootte, kort tydsduur, gevorderde fase van die infeksie en inter-toets variasie), is verdere navorsing benodig om die effektiwiteit van die voedingsupplement te bevestig.

ABBREVIATIONS

AI:	Adequate inadequate.
AIDS:	Acquired Immunedeficiency syndrome.
ARC:	AIDS-Related Complex.
BD:	Body density.
BMI:	Body mass index.
CCR:	Chemokine co-receptor.
CD:	Cluster differentiation.
cDNA:	Complementary DNA.
dl:	decilitre.
DNA:	Deoxyribonucleic Acid.
EDTA:	Ethylene diamine tetraacetic acid.
FDA:	Food and Drug Administration.
FFM:	Fat free mass.
FFQ:	Food frequency questionnaire.
FITC:	Fluorescein isothiocyanate.
ft:	Fetolitre.
gp:	Glycoprotein.
Hct:	Haematocrit.
HIV:	Human Immunodeficiency virus.
L:	Litre.
LBM:	Lean body mass.
MCH:	Mean cell haemoglobin
MCHC:	Mean cell haemoglobin concentration.
MCV:	Mean cell volume
MIP:	Macrophage inflammatory proteins
ml:	millilitre.
mm:	millimetre.
MRC:	Medical Research Council.
PBS:	Phosphate buffered saline.
PCR:	Polymerase reaction.
pg:	Picogram.
QS:	Quantitation standard.

RANTES:	Regulated upon activation normally T-cell expressed and secreted.
RDA:	Recommended daily allowances.
RDW:	Red distribution width.
REE:	Resting energy expenditure.
RNA:	Ribonucleic Acid
SKF:	Skinfold virus.
TEE:	Total energy expenditure.
Tris:	Tris (hydroxymethyl) aminomethane.
v/v:	volume/volume.
WCC:	White cell count.
WHR:	Waist-hip ratio.
µl:	Microlitre.

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CHAPTER 1

1. INTRODUCTION

1.1 General Background and Problem Statement

The HIV/AIDS epidemic continues to have a devastating effect on people in Sub-Saharan Africa. By the end of 1998 (Piwoz & Preble, 2000), at least 34 million people living in Sub-Saharan Africa had become infected with HIV and some 11.5 million of these have already died. In 1998 alone, about 2 million Africans died from HIV/AIDS (Piwoz & Preble, 2000). In global terms, South Africa is known to have one of the fastest growing AIDS epidemics. Approximately 1700 persons are believed to be infected with the HIV each day. It is also estimated that nearly 12% of adults are assumed to be HIV positive and 2.5 million will have died of AIDS, or an AIDS-related illness by 2005 (Dhianraj, 2000).

Malnutrition has been an endemic problem in Africa for decades, caused by a combination of factors, and more recently magnified by the impact of HIV/AIDS. HIV infection exacerbates malnutrition through its attack on the immune system and its impact on nutrient intake, absorption and utilization (Friis & Michaelsen, 1998). Nutritional deficiencies affect immune functions in ways that may influence viral expression and replication, further affecting HIV progression and mortality (Semba & Tang, 1999).

However, HIV-positive patients have demonstrated improved quality of life (increased activity, increased ability to perform activities of daily living and prolonged employment) after adequate provision of nutritional support (Futures Group, 1999). The impact of nutritional interventions mostly depend on the underlying nutritional and immune status of the individuals being studied (Watson, 1994) and many of the studies conducted on supplementation or nutritional support are in industrialised countries and may not be directly relevant to African settings.

Research is therefore urgently needed on the nutritional management of HIV/AIDS in Africa, where HIV infection is spreading very fast; where malnutrition is endemic, and where resources for management of both HIV and malnutrition are extremely limited. Studies are needed to determine the efficacy of multiple micronutrient supplements for improving nutritional status, slowing down HIV disease progression and delaying AIDS-related mortality in populations that are endemically deficient and may also experience chronic food insecurity. The quality of life and the prospects for millions of African adults and children living with HIV and AIDS today and those who are likely to be infected in the future may remain bleak if research into the nutritional management of HIV/AIDS patients is neglected.

Available scientific evidence has revealed that 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 (Tang *et al*., 1996; Chandra, 1997). Previous studies indicate that in the early period of HIV infection, weight gain or maintenance might be achieved through good nutrition and has helped to reduce the consequences of wasting in people living with HIV/AIDS (Watson, 1994; Semba & Tang, 1999; Piwoz and Preble, 2000).

Micronutrients have helped to strengthen the immune system and reduce the severity and impact of opportunistic infections in people living with HIV/AIDS (Watson, 1994; Semba & Tang, 1999; Piwoz & Preble, 2000). According to Piwoz & Preble (2000), the relationship between HIV/AIDS and malnutrition represents a classical instance of a well-recognised vicious circle of immune dysfunction, infectious disease and malnutrition. It is known that any immune dysfunction as a result of HIV/AIDS leads to malnutrition and this in turn leads to further immune dysfunction. This worsens the impact of HIV and may contribute to faster progression to AIDS. Various research studies have confirmed that nutrient deficiencies are associated with immune dysfunction and accelerated progression to AIDS (Baum & Shor-Posner, 1998; Fawzi & Hunter, 1998; Macallan, 1999; Bijlsma, 2001). For instance, deficiencies of vitamin A and B_{12} have been associated with a reduction in $CD4^{+}$T-cell count in HIV infected patients (Baum *et al.*, 1995).

Studies on nutritional supplementation in HIV positive patients are limited; while some targeted specific groups, others are more retrospective in nature or survey (Allard *et al.*, 1998; Nimmagadda, 1998; Muslimatun *et al.*, 2001). Furthermore, there have been some controversial reports on the benefits and/or role of nutritional supplementation in HIV/AIDS patients in general. Few studies (Vella *et al.*, 1995; Van Staden *et al.*, 1998; Tang & Smit, 1998) show a weak correlation between the dietary intake and blood levels of nutrients, which could lead to the conclusion that increasing intake of recommended macro-or micronutrients might not improve nutrient status. However, the weak correlation may be due to poor reliability or inaccuracy of intake measurement. In addition, studies have used a variety of different variables to characterize HIV status, making comparison between studies complicated. Research studies vary in their length of follow-up and may have biased conclusions. Other studies only measure changes in body weight following nutritional interventions whereas some measured body cell or lean body mass. Measuring changes in body weight and using this to measure the nutritional status of HIV infected subjects is less than optimal and may be misleading, because weight gain or stabilization can occur in the presence of muscle wasting when nutritional interventions increase body fat and water only. The differences in the study populations, study design and methodology have made it difficult to extrapolate findings from one population to another and to draw practical lessons for a specific and for different community settings. These reasons are a strong motivation for one to examine the role of nutritional intervention in HIV-infected patients in the African community of Bloemfontein.

Researchers have expressed the view that further and urgent community-based research studies are needed on nutritional supplementation among HIV positive patients (Fawzi & Hunter, 1998; Macallan, 1999; Kennedy *et al.*, 2001). Van Staden *et al.* (1998) reported a deficiency in several micronutrients in HIV-1 sero-positive patients in the Free State and suggested a multi-vitamin/anti-oxidant supplementation to improve the immune status of these patients. It is in line with his recommendations that the product Africa's Solution was devised. Observations by clinicians from preliminary results indicated that the product Africa's Solution increases the $CD4^{+}$T-cell count in HIV-infected patients. The viral load decreases over a period of time and the capacity for work of such people returns to normal. However, the product needs to be studied under research conditions.

1.2 Justification of the Study

Data on the prevalence of malnutrition, dietary intake and/or supplementation in HIV-infected persons in industrialised countries are widely available. However, this is often scarce in 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. A study concerned with nutritional supplementation of HIV positive patients could be considered as very important due to the points enumerated below:

- HIV infection frequently results in nutritional deficiencies and growth failure. The specific mechanism related to HIV infection, nutritional status and its role in human health is not well known.
- Benefits from nutritional supplementation include not only the improved health and well-being of individuals and families, but also improves the chances of prolonged survival for those infected.
- The knowledge acquired from this study would be vital in setting up nutrition intervention strategies for this group and other groups regarding various nutritional problems associated with HIV/AIDS.

1.3 The Aim of the Study

The aim of this study was to investigate the influence of a multiple combination liquid supplement on the $CD4^{+}$T-cell count and viral load of HIV-positive/AIDS patients with a depressed immune system, and to examine the change in the anthropometric status, haematological parameters and clinical conditions following supplementation for a given period.

1.3.1 Sub-aims

- To determine the dietary intake of the patients.
- To determine the effects of the supplement on anthropometric, haematological and immune parameters.
- To determine any correlation between the measured parameters at baseline and at the end of nutrient supplementation

1.4 References

Allard JP, Aghdassi E, Chau J, Tam C, Kovacs CM, Salit IE & Walmsley SL (1998). Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. **AIDS 12**: 1653-1659.

Baum MK & Shor-Posner G (1998). Micronutrient status in relationship to mortality in HIV-1 disease. **Nutr Review 51**: S135-S139.

Baum MK, Shor-Posner G, Lu Y (1995). Micronutrients and HIV-1 disease progression. **AIDS 9** 1051-1056.

Bijlsma M (2001). Nutritional care and support for people with HIV/AIDS: review of initiatives and recommendations for developing national programmes in sub-Saharan Africa. Working paper for WHO: 1-15.

Chandra RK (1997). Nutrition and the immune system: An introduction. **Am J Clin Nutr 66**: 4605-4635.

Dhianraj C (2000). Institutionalising the response to HIV/AIDS in the South African University sector: A SAUVCA analysis: 8-10.

Fawzi WW & Hunter DJ (1998). Vitamins in HIV disease progression and vertical transmission. **Epi 9**: 457-466.

Friis H & Michaelsen KF (1998). Micronutrients in HIV infection: a review. **Eur J Clin Nutr 52**: 157-163.

Futures group (1999). The impact of AIDS on various sectors. Paper presented at USAID's Consultative Meeting on AIDS as a development crisis: Rethinking strategies and results (published as a proceeding), Washington DC: 15-20

Kennedy OC, Coutsoudis A, Kuhn L (2001). Effects of vitamin A supplementation during pregnancy and early lactation on body weight on South African HIV-infected women. **J Health Popl & Nutr 19** (3): 167-176.

Macallan DC (1999). Dietary intake and weight loss patterns in HIV infection. In: Miller TI & Gorbarch SL, eds. Nutritional aspects of HIV infection. New York: Oxford University Press.

Muslimatunn S, Schmidt MK, Schultink W (2001). Weekly supplementation with iron and vitamin A during pregnancy increases haemoglobin concentration in Indonesian pregnant women. **J Nutr 13** (1): 85-90.

Nimmagadda H, O'Brien WA, Goetz MB (1998). The significance of vitamin A and carotenoid status in persons infected by HIV. **NIAID 29**: 74-76.

Piwoz EG & Preble EA (2000). HIV/AIDS and nutrition. A review of the literature and recommendations for nutritional care and support in sub-Saharan Africa: 1-22.

Semba RD & Tang AM (1999). Micronutrients and the pathogenesis of human immunodeficiency virus infection. **Br J Nutr 81**: 181-189.

Tang AM, Graham NMH & Saah AJ (1996). Effects of micronutrient intake on survival in HIV type 1 infection. **Am J Epidemiol 143**: 1244-1256.

Tang AM & Smit E (1998). Selected vitamins in HIV infection: A review. **AIDS Patient Care & STD 12**: 263-273.

Van Staden AM, Barnard HC, Nel M, Attwood EM, Dannhauser A, Van der Ryst E & Le Roux GD (1998). Nutritional status of HIV-1 seropositive patients in the Free State Province of South Africa-laboratory parameters. **Cent Afri J Med 44** (10): 246-250.

Vella V, Tomkins A, Nviku J (1995). Determinants of nutritional status in South-west Uganda. **J Trop Paedictr 41**: 89-98.

Watson RR (1994). Nutrition and AIDS. 2^{nd} Edition, CRC Press, London: 1-30.

2 LITERATURE REVIEW

2.1 Introduction

The human immunodeficiency virus (HIV) is seen as one of the most severe infections ever known to have attacked the human population, especially the economically productive age group of between 15-49 years (UNAIDS, 2001). HIV is also seen as an infection of attitude and behaviour, as it is closely associated with sexual behaviour, especially where a person has more than one sexual partner (UNAIDS, 2001; Oguntibeju & Fabode, 2002). Scientific evidence has shown that HIV infection is caused by a retrovirus named the HIV. HIV is a ribonucleic acid (RNA) retrovirus, so designated because of its genome that encodes an unusual enzyme called reverse transcriptase. This enzyme allows deoxyribonucleic acid (DNA) to be transcribed from RNA. Thus HIV can make copies of its own genome as DNA in the host's cells, such as human T4-helper lymphocytes, and this leads to the elaboration of vast numbers of viral particles (Weiss, 1996; Denny *et al.*, 1998; Oguntibeju & Banjoko, 2003). There has been a drastic increase in the number of people infected with HIV, in spite of various efforts made to combat this infection. This increase is not peculiar to a particular racial group, country and community. It is a worldwide problem. However, according to reports, the greatest incidence of HIV infection is found in Sub-Saharan Africa (UNAIDS, 2001; SAHIVCS, 2001).

2.2 Definition of AIDS

The acquired immunodeficiency syndrome (AIDS) is caused by a retrovirus named the HIV which selectively attacks and depletes T-lymphocytes bearing the CD4 receptor (T-helper cells), causing a predisposition to opportunistic infections and malignancies (Sabatier, 1987; Weiss, 1996; Wong *et al.*, 1997). In its definition, the World Health Organization (WHO) gave a provisional clinical case definition for AIDS in places where diagnostic resources are limited, and stated thus:

2.2.1 AIDS in Adults

This is defined by the existence of at least two of the major clinical signs associated with at least one minor clinical sign in the absence of known causes of immuno-suppression such as cancer or severe malnutrition, or other recognised aetiologies. The major clinical signs include weight loss of more than 10% of the body weight, chronic diarrhoea for more than one month and prolonged fever for longer than one month. The minor signs are generalised lymph-adenopathy, a persistent cough for longer than one month and oropharyngeal candidiasis. It also stated that the presence of generalized Kaposi's sarcoma or cryptococcal meningitis are sufficient clinical signs by themselves for the diagnosis of AIDS in the adult group (WHO, 1993; Barnhart *et al*., 1996; SAHIVCS, 2001).

2.2.2 AIDS in Children

Paediatric AIDS should be suspected in an infant or a child presenting with at least two major clinical signs associated with at least two minor signs in the absence of known causes of immuno-suppression. The major clinical signs include weight loss or abnormally slow growth and chronic diarrhoea for longer than one month. The minor clinical signs include generalised lymph-adenopathy and oropharyngeal candidiasis (WHO, 1993; Barnhart *et al*., 1996; SAHIVCS, 2001).

2.3 Natural History of HIV Infection

Observations have shown that from the natural history of the disease, it is usually lethal to those who are infected by HIV. Furthermore, every person infected is an active carrier of the virus to an unsuspecting contact, even before the infected carrier presents with AIDS. The mean time from infection with HIV to the development of AIDS is about nine to ten years in industrialized countries (Weiss, 1996; Liu *et al*., 2000). Factors that are suggested to affect the duration of clinical latency include genetic susceptibility, viral load, concurrent infections as well as the pre-existing immune status at the time of HIV infection (Royce *et al*., 1997). Based on available data, the survival time of HIV infected persons is much shorter in Africa than in industrialised countries (Enwonwu, 1992; Gilks, 1993; Piwoz & Preble, 2000; SAHIVCS, 2001).

Studies suggest a rapid transition from asymptomatic HIV infection to AIDS (Enwonwu, 1992; Bentwich *et al.*, 1999; Hazenberg *et al.*, 2000). Many of the patients in Africa have poor access to health care systems, therefore infections due to endemic pathogens such as *Mycobacterium tuberculosis*, *Streptococcus pneumonia* and non-typhoid salmonellae coupled with malnutrition seem to be playing a significant role in the cause of death (Gilks, 1993; Tindall & Copper, 1991; SAHIVCS, 2001).

2.3.1 Classification of HIV Infection

In 1986, the United States Centers for Disease Control and Prevention (CDC) developed the first classification system for HIV infection with the focus on AIDS-defining opportunistic infections and malignancies (CDC, 1987). The CDC classification system was revised in 1987 to reflect clinical practice more practically and included in it the "HIV wasting syndrome" along with other HIV related diseases. The former classification refers to the presence of involuntary weight loss of greater than 10%, accompanied by chronic diarrhoea (> 30 days) or documented fever (> 30 days). In 1991, the classification of AIDS included all HIV-infected individuals with $CD4^+$ T-cell counts < 200 x 10^6 (Buehler *et al.*, 1993; SAHIVCS, 2001). In 1993, the WHO staging system for HIV infection and disease was formulated as universal staging system for HIV infection with some flexibility for international use, including countries with limited access to technology. The WHO did not include pulmonary tuberculosis as an AIDS defining illness within this staging system, since it occurs as an endemic disease in many developing countries. Involuntary weight loss of greater than 10% of normal weight in clinical stage 3 and the HIV wasting syndrome in stage 4 were included (WHO, 1993; SAHIVCS, 2001). This staging is currently in use in South Africa (Martin 2000, SAHIVCS, 2001).

2.4 The Epidemiology of HIV/AIDS

2.4.1 Origin and History of HIV/AIDS Epidemic

The first reported cases of AIDS were recorded in the morbidity and mortality weekly report of June 5 1981, in the United States of America and concerned five young men, all active homosexuals who had been treated in Los Angeles hospital for a rare infection called Pneumocystis carinii pneumonia (PCP). All five men had evidence of other infections and a defective immune system. About the same period, physicians in the United States of America diagnosed Kaposi's sarcoma (an uncommon malignancy) in twenty-six homosexual men whose ages ranged from 26-51 years (Cahill, 1994; UNAIDS, 2000; 2001).

2.4.2 Global Epidemiology of HIV Infection and AIDS

The HIV/AIDS pandemic is perhaps the most serious disease threat to this generation. There is, however, inadequate information on the extent of its spread (UNAIDS, 2002). Reports show that global statistics are collated by WHO, but these statistics are usually incomplete because, for a number of reasons, many authorities do not/are unable or are unwilling to give an accurate figure regarding the number of cases occurring in their countries (Sabatier, 1987; SAHIVCS, 2001). The epidemiological data available on the distribution of HIV/AIDS cases throughout the world point to distinct patterns, each of which is characterized by the time the infection or disease was diagnosed and the predominant modes of transmission (Martin, 2000; UNAIDS, 2002).

At the end of 2001, an estimated 40 million people globally were documented to be living with HIV. In many parts of the developing world, the majority of new infections occur in young adults, with young women especially being more vulnerable. About one-third of those currently living with HIV/AIDS are between 15 and 49 years of age. Most of them do not know that they are carrying the virus. Many people in Africa especially those living in the rural areas, know nothing or too little about HIV to protect themselves against the virus (UNAIDS, 2001; Oguntibeju *et al.*, 2002).

The pattern of HIV/AIDS distribution has implications for the type of prevention and nutritional support needed and for the priority that needs to be given to different aspects of the activities that would reduce its spread and improve the health status of those already infected with HIV (UNAIDS, 2000).

2.4.2.1 Pattern 1

In pattern 1 areas, most cases of HIV infection and AIDS occur in homosexual or bisexual males and in intravenous drug users. Heterosexual transmission is also increasing, but it is responsible for only a small percentage of cases. This pattern is typical of North America. The male to female ratio of HIV/AIDS reported cases range from 10:1 to 15:1 (Royce *et al.*, 1997; UNAIDS, 2001).

2.4.2.2 Pattern 2

This is typical of most Sub-Saharan Africa and parts of the Carribbean. Most of the reported cases in these regions occur through sexual transmission among heterosexuals with the male to female ratio of documented cases put at ratio 1:1. Transmission from intravenous drug users and homosexual relationship occurs at a lower rate than heterosexual transmission (Royce *et al.*, 1997; UNAIDS, 2000).

2.4.2.3 Pattern 3

This pattern is found in North Africa, Eastern Europe, Asia and most parts of the Pacific. In these areas, HIV appears to have been introduced in the early to mid 1980s and only a small number of HIV/AIDS cases have been documented as of 1989. However, the prevalence rate of HIV/AIDS in the different regions has increased significantly in the last few years (Royce *et al.*, 1997; UNAIDS, 2000).

2.5 HIV Epidemics in Sub-Saharan Africa

Africa is believed to be home to at least 70% of the total adults and 80% of the total children globally living with HIV (Piwoz & Preble, 2000; UNAIDS, 2002). About 75% of all AIDS deaths have occurred in sub-Saharan Africa since the epidemic began two decades ago. At the end of 2001, it was reported that of the 40 million people estimated to be living with HIV/AIDS, 28.5 million live in Sub-Saharan Africa. Of approximately 13.2 million children who have lost both parents due to HIV infection, 12.1 million live in Sub-Saharan Africa (UNAIDS, 2001; WHO, 2002). Available data showed that countries in southern Africa now have prevalence rates higher than 20%: Botswana (38.8%), Lesotho (31%), Namibia (22.5%), South Africa (20.1%), Swaziland (33.4%), Zambia (21.5%), and Zimbabwe (33.7%) (Maw, 2000; WHO, 2001).

In West and Central Africa, new data confirm an increased rate. In Cameroon's urban areas, the HIV prevalence rate increased from 2% in 1988 to 4.7% in 1996. Nigeria's national prevalence rate rose from 1.9% in 1993 to 5.8% in 2001. Already about 3.5 million Nigerians are estimated to be living with HIV/AIDS (UNAIDS, 2001; 2002). The rise of HIV prevalence in conflict countries such as Angola, Burundi, the Democratic Republic of Congo and Rwanda is considered a serious concern as the extensive displacement of people and the disruption of social systems increase the vulnerability of the people to HIV infection (UNAIDS, 2001; 2002). Table 2.1 reveals a global summary of the HIV/AIDS epidemic.

Table 2.1: Global Summary of HIV/AIDS Epidemic, December 2001 (UNAIDS 2001)

Number of people living with HIV/AIDS	Adults Men Women Children under 15 years	37.2 million 16.9 million 17.6 million 2.7 million
People newly infected with HIV in 2001	Adults Men Women Children under 15	4.3 million 1.7 million 1.8 million 8 00, 000
AIDS deaths in 2001	Adults Men Women Children under 15 years	2.4 million 720 000 1.1 million 580, 000

2.5.1 Prevalence of HIV/AIDS in South Africa

In global terms, South Africa is known to have one of the fastest growing AIDS epidemics. Approximately 1700 people are believed to be infected with HIV each day (Dhianraj, 2000). The HIV-1 epidemic in South Africa started in the white homosexual population in the mid-1980s. However, by 1990 it was clear that heterosexual contact was becoming the predominant mode of transmission in the country, with a subsequent explosive epidemic occurring in the heterosexual population (ADSA, 1996; Dannhauser *et al.*, 1999).

The HIV/AIDS epidemic in South Africa continues to grow at a rapid rate. UNAIDS estimates that in 2000, 19.9% of adults South Africans were infected, exceeding the 12.9% recorded in 1998 (UNAIDS, 2000). Reliable empirical data on the HIV epidemic in South Africa as in other African countries are, however, difficult to obtain (Dorrington *et al.*, 2001). According to Dorrington *et al.* (2001), the death rates increased from 9% in 1995 to 40% in 2000.

2.6 Modes of Transmission of HIV

HIV is present in peripheral blood, cell free plasma, semen, cervical and vaginal secretions, lymphnodes, brain cells, liver cells, cerebrospinal fluid (CSF) and saliva (Sabatier, 1987; DeGruchy, 1990; Soderlund *et al.*,1999; SAHIVCS, 2001). HIV can be transmitted via

- Sexual acts (horizontal transmission).
- Blood or blood products from an infected person transfused to another individual.
- Intravenous drug abuse

Needle stick injury and other parenteral modes of innoculation of the virus as well as ear piercing, tribal marks and scarifications are other methods of transmission (DeGruchy, 1990; SAHIVCS, 2001). In addition, Royce *et al.* (1997), reported that HIV can still be transmitted by people receiving highly active anti-retroviral therapy (HAART) and/or by those who have undetectable viral loads. HIV-1 and HIV-2 are transmitted in the same ways, although there is evidence that HIV-2 is less easily transmitted through the sexual route and mother to child route than HIV-1 subtypes.

Epidemiological evidence showed that HIV-1 subtypes seem to be predominant in certain epidemics (for example, the rapid heterosexual spread of subtype E strains in Thailand). However, there is no clear evidence to link particular subtypes with a specific mode of transmission (DeVicenzi, 1994; Bartlett, 1998; Gallant, 1999).

2.6.1 Sexual Transmission

As Royce *et al.* (1997) stated, unprotected sexual intercourse (defined as penetrative oral, vaginal or anal sex) without the use of a condom between a male and a female or between males, accounts for 75-85% of HIV infection in adults. One of the most complex factors affecting the pathogenesis of HIV infection is that of sexual transmission. Sexual transmission of HIV is dependent on a number of factors that are involved in both the person transmitting the virus and the uninfected partner (Darby *et al.*, 1996; Denny *et al.*, 1998; Oguntibeju & Fabode, 2002; Oguntibeju *et al.*, 2002). Sexual transmission of HIV may occur when a sufficient amount of the infectious virus penetrates the mucosa of an individual during sexual relations (Denny *et al.*, 1998).

It has been suggested that factors that increase the amount and virulence of the immunodeficiency virus; weaken the integrity of the localised tissue barriers, or interrupt with the production of an effective local and systemic immunological response, may increase the chances of transmission of HIV (Denny *et al.*, 1998; Cohen & Fauci, 2001). Seidlin *et al.* (1993); Weiss (1996); Royce *et al.* (1997); Denny *et al.* (1998); UNAIDS (1999); Wawer *et al.* (1999) and Maw (2000) noted that several factors are creating a fertile ground for the epidemic. These factors include host susceptibility, host genetic factors, stage of infection, rate of partner change, the biological property of HIV, mass unemployment and economic insecurity, social and cultural norms, other sexually transmitted infections and unprotected/unsafe sex.

2.6.2 Blood-borne Transmission of HIV

Generally, blood-borne transmission of HIV occurs via needle sticks, other blood-contaminated sharp objects and via blood transfusion and organ transplantation. The people at risk are intravenous drug users, healthcare workers and recipients of blood, blood products and organs (Cardo et al., 1997). Globally, by the end of 1996, blood transfusion accounted for 3-5% of all adult cases of HIV infection (Efem, 1990; Cardo *et al.,* 1997; Leroy *et al.*, 1998). Routine screening procedures have significantly reduced this risk, especially in developed countries. However, there are reported cases of HIV having been transmitted through transplantation of blood-containing or highly vascularised organs such as the kidney, liver, heart, pancreas, bone, skin and via artificial insemination (Efem, 1990; Smith & Nichols, 1991). Healthcare workers are continually at risk of acquiring blood-borne infections, in particular Hepatitis B and C viruses and HIV (Bragbjerg, 1993).

2.7 The Biology of HIV

There are two types of HIV which show approximately 40-60% amino acid homology and these two types are discussed below.

2.7.1 Type 1 (HIV-1)

Type-1 is found throughout the world and is responsible for the majority of cases of HIV infection (Fox, 1996; SAHIVCS, 2001). HIV-1 strains are further divided into group M (major) and group O (outlier) strains. Group M viruses are a prevalent group accounting for most HIV-1 infections worldwide. On the other hand, the smaller group O strains are rare and are quite diverse from the group M viruses (Kuritzkes, 1999). The reason for the genetic diversity of HIV-1 is related to the inherent potential of the virus to mutate and in some instances is due to the recombination of distinct virus strains.

The initial epidemic in South Africa was due to a clade B virus, but this has been overtaken by a clade C epidemic (Martin, 2000). It was confirmed that each HIV particle is composed of two copies of the single-stranded RNA viral genome packaged inside a protein core or capsid. The core (Gag) proteins include the major structural proteins p24 (capsid) and p17 (matrix), the internal structural protein p7 (nucleocapsid) and the Gag-Pol precursor protein p55. The virus particle also contains polymerase (Pol) proteins that are essential for the early steps in the life cycle of the virus. This includes the reverse transcriptase p66/p51 and the endonuclease/integrase p31 (P[illegible] et al., 1998). The capsid of HIV is surrounded by lipid envelope derived from the infected cells, in which HIV envelope glycoproteins (Env) are embedded. These comprise the outer envelope glycoprotein gp120, the transmembrane glycoprotein gp41 and the precursor glycoprotein gp160 (Vogt *et al*., 1997; Martin, 2000).

2.7.2 Type 2 (HIV-2)

Type 2 is currently and predominantly found in West Africa and countries with historical or commercial ties to this region (Efem, 1990, Fox 1996). HIV-type 2 was isolated first in 1986 from AIDS patients in West Africa where it is most prevalent and mainly acquired through heterosexual relationships. The incidence of HIV-2 has also been reported from East Africa, Europe, Asia, North America and Latin America. Five HIV-2 sub-types (A-E have been described (Moore *et al*., 1997; Young, 1997; Kuritzkes, 1999; Martin, 2000).

2.7.3 Cellular Receptors for HIV

HIV is known to infect certain types of cells; these are cells expressing the $CD4^+$T-cell receptor. These cells include T-helper cells ($CD4^+$ T-cells or T4 cells), as well as other white blood cells, including monocytes and macrophages. Glial cells in the central nervous system, chromaffin cells in the intestine and Langerhans cells in mucous membranes and skin that express the $CD4^+$T-cell receptor can also be infected with HIV (Bagasra *et al*., 1992; Paxon *et al*., 1996).

However, some cells, for example, neurones that do not express the $CD4^+$T-cell receptor, may become infected with HIV. This raises the possibility that other cellular targets may exist for the human immunodeficiency virus. Research findings indicate that specific human cell surface proteins identified as co-receptors in addition to the $CD4^+$T-cell, have been found to mediate fusion between HIV and its target cells (Paxon *et al*., 1996; Grossman & Herberman, 1997). Two of the prominent co-receptors are: C-C chemokine receptor CCR-5, expressed by monocytes and lymphocytes which mediate entry of non-syncytium inducing (NSI), monocytotropic strains of HIV-1, and the C-X-C chemokine receptor CXCR-4 (also known as fusin)-

expressed on T-lymphocytes wh[illegible]y of syncytium-inducing (SI), T-cell tropic strains of HIV-1. This possibly explains the reason why monocytotropic strains of HIV can infect both monocytes and primary (both of which express CCR-5) but not T-cell lines (which lack CCR-5 and why T-cell tropic strains of HIV-1 can not infect monocytes (which lack CXCR-4). The CCR-5 and CXCR-4 co-receptors function as receptors for chemokines in the human body system.

It has been postulated that chemokines that are produced by $CD8^+$ cells in response to immune activation, such as RANTES (regulated upon activation, normally T-cell expressed and secreted), MIP-1α and MIP 1β (macrophage inflammatory proteins), bind to CCR-5, blocking the entry of HIV-1, thereby inhibiting *in vitro* infection with monocytotropic but not T-lymphotropic strains of the virus (Cocchi *et al.*, 1995; Paxon *et al.*, 1996; Ullum *et al.*, 1998).

2.8 Pathogenesis of HIV Infection

2.8.1 Introduction:

Most characteristically, HIV-1 infection in man results in the loss of function and death of $CD4^+$T-cells with a resultant increased incidence of opportunistic infections and malignancies (Levy, 1993; Fox, 1996). Patients that are seropositive for HIV-1 positive, progress through the infection at different rates and factors that affect the progression are clues to the pathogenesis of HIV-infection (Schechter *et al.*, 1994; Dalgleish & Colizzi, 1992; Levy, 1993; Kreiss *et al.*, 1997). Literature tends to agree that genetic predisposition, reflected in different human leucocyte antigen (HLA) types, concurrent infections, age, dose of the inoculum, the route of exposure, the variant of the virus as well as other factors, plays a role in the pathogenesis of HIV-1 and in determining how the immune system handles the infection.

2.8.2 Processes of Pathogenesis of HIV Infection

The sequence of events associated with the processes of pathogenesis by Fox (1991); Fox *et al.* (1992); Fox & Cottler-Fox, (1992); Fox *et al.* (1994); Fox, (1996) can be summarized as follows:

- HIV, covered with cel tibodies, and/or complement from the infected individual enters into a new host.
- The infecting virus is immunologically recognized as an "antigen complex" and as such is attacked by CD4$^+$T-cells. Contact with protruding gp120 results in infection of the host cells.
- The infected cell produces many more viral particles that are not coated with antibodies and are therefore able to freely produce infection among CD4$^+$ bearing cells.
- Infected virus-producing cells shed antigens in the form of virions and viral proteins. These substances are immunogenic.
- There is lymphoid hyperplasia accompanied by B-cell expansion and increasing levels of humoral antibodies and plasma cells. This is followed by a proportionate decrease in the numbers of circulating productively infected cells. This reciprocal change in virus-producing cells may either be due to cytotoxic T-lymphocytes, or to virus inactivation by humoral antibodies or both.
- There is an accumulation of HIV as a viral-immune complex on the surface of the cell membranes of follicular dendritic cells. This is attached by Fc receptors on the cell surface. The virus reservoir is required for the progression of HIV infection and viral concentration of 1x10^9 particles per cubic centimetre may be reached in the germinal cells.
- The infected individual assumes a steady state of infection in which the CD4$^+$ T-cells become infected as they migrate through the germinal cells. It is said that depending on the time from infection to activation and viral expression, infected cells produce viral particles in the lymphoid tissue or at distant sites elsewhere in the body system.
- The depletion of CD4$^+$T-cells exceeds formation by a slight margin. This phase of the infection/disease can continue for many years.
- Due to the prolonged loss of CD4$^+$T-cells and their functional interactions, the integrity and function of the lymphoid tissues are broached and there is loss of filtration of virus/virions as well as general disorganization of the lymph nodes. Lymphoid tissues seem to depend on complex ecological interactions, modulated by cytokines and dependent on micro-

environmental interaction. At some point in this process, the AIDS-defining stage is reached.

- Immune function decreases until opportunistic AIDS-defining infections overwhelm the patient.

2.8.3 Principles of HIV Pathogenesis

Infection with HIV-1 initiates a process that leads to progressive destruction of the population of CD4$^+$T-cells with roles in the generation and maintenance of host immune responses (Fauci, 1988; Fauci *et al.*, 1991; Feinberg, 1995; Koot *et al.*, 1996; Haynes *et al.*, 1996). The target cell preference for HIV-1 infection and depletion is determined by the identity of the CD4$^+$T-cell surface that is recognized by the HIV-1 envelope (env) glycoprotein as the virus binds to and enters host cells to initiate the virus replication cycle (Feinberg, 1995; Koot *et al*, 1996). It has been found that the process of cell fusion (syncytium formation) which depicts a significant cytopathic consequence of HIV-1 infection of CD4$^+$ T-cells, also depends on the specific interaction between CD4$^+$T-cells and the HIV-1 env glycoprotein (Feinberg, 1996; Bartlett, 1998).

2.8.3.1 Decline of CD4$^+$T-cells

After initial infection of the human host, the pace at which immunodeficiency develops and the susceptibility to opportunistic infections and malignancies become manifest and are associated with the rate of decline in CD4$^+$T-cell levels (Stein *et al.*, 1992; Kaplan *et al.*, 1995; Enger *et al.*, 1996; USPHS/IDSA, 1996). The rate of CD4$^+$T-cell decline varies considerably from person to person and is not constant throughout all stages of HIV-infection. Koot *et al.* (1996) reported that acceleration in the rate of decline of CD4$^+$T-cell numbers heralds the progression of the disease. According to Koot *et* al. (1996), the virological and immunological processes that take place during the point of onset of a rapid fall in CD4$^+$T-cell count are poorly understood. However, they are believed to be associated with increasing rates of HIV-1 replication *in-vivo* and declining cell-mediated immune response.

2.8.3.2 Progression of HIV to AIDS

In developed countries, the average time for adults to develop AIDS after initial HIV-1infection is about 10-12 years in the absence of antiviral therapy. However, some individuals (20%) manifest full-blown AIDS within five years of infection, whereas others (<5%) have sustained long-term (>10 years) asymptomatic HIV-1 infection without significant decline in $CD4^+$T-cell counts. As Haynes *et al.* (1996) observed, about 2% of HIV-1 infected persons seem to be able to contain viral replication to extremely low levels and maintain stable $CD4^+$T-cell counts within the normal range for lengthy periods (12-15 years). Also, within this group, very rare individuals are infected with HIV-1 variants harbouring genetic defects. Most instances of slowly progressive or apparently non-progressive HIV infection are believed to result from more effective host antiviral immune responses. These individuals tend to have active reactive cytotoxic T-cell responses against HIV-1 infected cells (Haase, 1999).

2.8.3.3 Inheritance of Specific Gene

Inheritance of a specific gene other than the HLA gene has also been implicated in the rate of progression of HIV-1 disease. Researchers are uncertain as to whether the $CD4^+$T-cells from different individuals vary in their susceptibility to the cytopathic effect of HIV-1 infection. However, few people who are not infected with HIV-1 despite multiple exposures to the virus, display higher levels of β-chemokine production and altered expression of the specific chemokine receptors, for instance CCKR-5 that serve as co-receptors for entry to HIV-1 into target cells (Paxon *et al.*, 1996; Pakker, 1998).

2.8.3.4 Age

Stein *et al.* (1992) and Darby *et al.* (1996) reported that the age of people living with HIV-1 infection could influence the rate of progression to AIDS and this suggests that the regenerative capacity of the host immune system (known to decline with age) may equally determine the integrity of the immune system (how it resists or repairs the damage caused by HIV-1 infection).

2.8.3.5 Environmental Facto_ _

Environmental factors, particularly the ones leading to activation of the immune system, may affect the rate of HIV-1 induced immunosuppression (Ho *et al.*, 1995; Hu *et* al., 1996; Oguntibeju *et al.*, 2002). Exposure to environmental antigens may activate HIV-1 replication, thereby increasing immune damage and enhancing progression of HIV-1 infection (Ho *et al.*, 1995). The authors also believe that current HIV-1 replication in the presence of an active but incompletely effective host antiviral immune response may partly be responsible for secondary manifestations of the HIV-1 disease. Similar to the above, Kuritzkes (1999) and MacDougall (1997) argued that viral attributes such as cytopathicity, replicability, syncytiality, cell tropism, virulence and viral load, apoptosis, antigenic dominance or competition, induced or random viral escape mutants, autoreactive cells of $CD8^+$ or $CD4^+$ phenotypes and inhibition of the production of pre-cursor cells, are sufficient to explain the pathogenesis of HIV.

2.9 The Immune System

2.9.1 Introduction

The healthy human body continually comes into contact with micro-organisms and other environmental assaults capable of causing diseases. Despite these constant challenges, infection and diseases are usually prevented (Rice, 2000). The mechanism by which the body protects or defends itself from these environmental assaults constitutes the body's immune system (Cheesbrough, 1992; Haase, 1999).

2.9.2 Classification of Immune System

There are two types of immune responses: non-specific and specific. Specific immune responses are further divided into two types: cell-mediated and humoral (Turk *et al.*, 1993; Feinberg, 1995). The immune system, although classified into natural immunity and acquired immunity for the purpose of clarity, are not separate systems working on their own. Both work together with some interaction, for example, macrophages present antigen to the T-lymphocytes and in turn trigger B-lymphocytes to produce antibodies and other specific lymphocytes (Cheesbrough, 1992; Bartlett, 1998).

2.9.2.1 Natural Immunity

Natural immunity is provided by all the mechanisms that form an immediate non-specific barrier to infection. They consist of mechanical barriers, chemical barriers, phagocytosis and antimicrobial commensal flora (Turk *et al.*, 1993; Fox, 1996; Koot *et al.*, 1996).

2.9.2.2 Acquired Immunity

Acquired immunity refers to all the mechanisms that are involved when the body actively responds to a foreign substance such as an infecting organism. This specific immunity has the hallmark abilities of learning, adaptability and memory. It learns to recognize a foreign body, adapt to response and remembers it, as it were, for a much more rapid response when next it encounters the same micro-organism (Cheesbrough, 1992; Turk *et al.*, 1993). The first immune response may take some 2-3 weeks and in some severe diseases death may occur within that time. The subsequent responses are usually rapid within that period of time. Studies have shown that acquired immunity is composed of two different systems, namely:

(i) Humoral (soluble) immunity

(ii) Cellular (cell-mediated) immunity

Both systems rely on the body's ability to differentiate between self (part of the body) and non-self (foreign substances), determined by major histocompatibility and relies on the supply of stem cells from the bone marrow (Cheesbrough, 1992; Nowak , 1997).

(i) Humoral Immune Response

The response of the humoral system to a non-self antigen is complex and requires co-operation between macrophages (antigen presenting cells), T-lymphocytes (regulatory cells) and B-lymphocytes (producers of antibody). B-lymphocytes have a certain antibody (immunoglobin) on their surface. When a non-self antigen associated with macrophages a [illegible]; meets a B-lymphocyte with the corresponding specific antibody, the antigen will combine with this antibody (Cheesbrough, 19992; McCune, 2001). A rapid cell division then takes place in which more identical B-lymphocytes are formed and plasma cells are formed that produce and release specific antibodies into the blood stream to react with the non-self antigen. Each plasma cell produces one type of antibody (Cheesbrough, 1992; Turk *et al*., 1993; Weir, 1993).

(ii) Cellular Immunity

This is mediated by T-cells that mature, acquire functional repertoires and learn the concept of self in the thymus. They have antibody-like binding sites on their cell surface that bind specifically with non-self antigen (Cheesbrough, 1992; Turk *et al*., 1993). Cellular immunity and T-lymphocytes are mainly involved in reactions against non-self antigen on or in body cells, for instance they react with cells that contain micro-organisms. This is particularly important in viral infections (Weir, 1993; McCune, 2001).

2.9.3 The Immune Response to HIV-1

The major factor obstructing progress towards an effective vaccine to prevent or modulate HIV-1 infection is that the critical features needed for a protective immune response are not fully understood (Martin, 2000). Although, it has been found that potent neutralizing antibodies can protect against experimentally acquired HIV infection in animal models, they are scarcely generated *in vivo* in the infected person and neutralization resistant viral variants have been noticed to develop rapidly in chronic infection. It is generally believed that cellular immune responses, particularly specific cytotoxic T lymphocytes (CTL), are important in the host response to HIV-1 infection (Paxon *et al*., 1996). Paxon *et al*. (1996) observed that CTL develop very early in acute HIV-1 infection, coincident with a rapid fall in plasma vireamia, whereas in chronic infection their levels are inversely related to viral load. However, the powerful HIV-specific CTL response ultimately fails to control HIV replication. This could be due to the emergence of viral variants that escape CTL recognition or impairment of CTL function (Allen, 2000).

2.9.3.1 Progressive Destruction of the Immune System in Advanced HIV Infection

In the absence of suppressive antiviral therapy, HIV infection advances and progressively infects more cells in the follicular dendritic cell (FDC) network, resulting in the destruction of lymph node architecture. This promotes the release of more viral particles into the circulation (SAHIVCS, 2001). The release of HIV into circulation causes $CD4^{+}$T-cells to be infected and destroyed at a faster rate than they can be replaced, thereby shifting the dynamic equilibrium in favour of the virus (Wei *et al.*, 1993; Ho *et al.*, 1995; Chun, *et al.*, 1997). Recent studies have shown that an important cause of declining $CD4^{+}$T-cell counts is the failure of the regenerative capacity of the immune system to produce immune cells (Ho *et al.*, 1995). This is a result of HIV infection of precursor stem cells and is also due to failure of "programming" of $CD4^{+}$T-cells in the HIV-infected thymus gland. This culminates in the destruction of the immune system and consequent failure to mount immune responses that are adequate to prevent opportunistic infection and/or neoplasmic disease (Wei *et al.*, 1993; Ho *et al.*, 1995; Roederer, 1995).

2.9.4 $CD4^{+}$T-cell and its Significance

2.9.4.1 Introduction

$CD4^{+}$ is a molecule found primarily on the surface of helper T-lymphocytes. The designation CD stands for "Cluster of Differentiation" and refers to a nomenclature applied by immunologists who are all involved in the business of generating monoclonal antibodies against surface proteins of blood cells as a means of identifying the surface proteins and studying them. When a "cluster " of monoclonal antibodies (antibodies produced *in-vitro* by single clones of B-lymphocytes) is found to react with the same protein, it represents a group of reagents defining a specific marker and that marker is given a CD number (SAHIVCS, 2001).

2.9.4.2 Functions of CD4$^+$T-ce

The CD4$^+$ molecule is also the main receptor involved in combating HIV-1 infection and docks with the 120 glycoprotein found on the envelope of the HIV-1 viral particle. The CD4$^+$T-cell has a central and coordinating role in the immune response (Hughes *et al.*, 1997). These cells, also known as T4 or helper/inducer T lymphocytes, recognize antigens presented by cells bearing HLA class 11 molecules such as monocytes and macrophages. The CD4$^+$molecule helps to stabilize the binding of these T-lymphocytes to the HLA 11 molecule on the antigen-presenting cell (Wilson, 1990). Once an antigen is recognized, CD4$^+$T lymphocytes orchestrate the body's antigen-specific immune response. Specific functions of CD4$^+$ T lymphocytes include the following: (1) coordinating B-lymphocyte production of antibodies to these antigens; (2) producing cytokines, and (3) induction of cytotoxic lymphocytes. These functions make CD4$^+$ T lymphocytes critical elements of the immune system and their dysfunction and destruction in HIV-1 infection seriously impairs the ability to respond to diverse pathogens (Bowen *et al.*, 1995; SAHIVCS, 2001).

2.9.4.3 Dynamic Equilibrium between CD4$^+$T-cell Production and Clearance

The initial process in the life cycle of HIV infection is the binding of HIV glycoprotein (pg120) that is present on the surface of the virus to CD4$^+$ molecule (Dalgleish & Colizzi, 1992; McDougal *et al.*, 1996; Bentwich *et al.*, 1999). Once the virus gains entry into the cell, it begins the process of viral replication following binding to CD4$^+$T-cell and fusion with the cell membrane. A direct cytopathic effect of HIV on CD4$^+$T-cells may occur via the destruction of the cell membrane that is subsequent to massive viral budding (Rowland-Jones *et al.*, 1997; Denny *et al.*, 1998), the presence of large amounts of non-integrated viral DNA, heterodisperse RNAs and viral core proteins in the cytoplasm of the infected cell that interferes with proper cell function, and the complexing of HIV gp120 with intracellular CD4$^+$ molecules (Rosenberg and Fauci, 1990; Weiss, 1996; Hughes *et al.*, 1997).

The depletion of CD4$^+$T-cells may also be due to the destruction of precursor cells that give rise to CD4$^+$T-cells *in vivo* or cells which produce factors essential for CD4$^+$T-cell growth (Rosenberg & Fauci, 1990). An alternative mechanism for CD4$^+$T-cell depletion involves the forr /ed multinucleated giant cells or syncytia between an HIV-infected CD4$^+$T-cell and a cluster of uninfected CD4$^+$T-cells. It has been estimated that between 100 million and 10 billion virus particles are produced and cleared every 24 hours. Thus, virus production occurs not only during the primary and advanced stages of the disease (typically symptomatic) but also during the intermediate stage of the infection (the virus is not truly latent) (D'Souza & Haden, 1996; Wong *et al.*, 1997). At the intermediate stage of HIV infection, levels of the virus in the plasma are the result of a dynamic equilibrium between the production of HIV and its clearance by the immune system. In this continuous turnover of the virus population, about 50% of the circulating virus is suggested to be replaced with newly produced virions daily (Young, 1997). The extensive production of HIV is closely related to the destruction and replacement of CD4$^+$T-cells. Scientists have estimated that the half-life of HIV-producing CD4$^+$T-cells is about 1.2 days and between 1-2 billion new CD4$^+$T-cells are produced every day (Pantaleo *et al.*, 1998). There appears to be a difference between the half-lives of various populations of HIV-infected cells. It is said that productively infected CD4$^+$T-cells constitute a short-lived population (about 1.2 days), while other HIV-infected cells, for instance macrophages, constitute a long-lived population with an average half-life of 14 days (Young, 1997; Wong *et al.*, 1997; Pantaleo *et al.*, 1998).

2.9.4.4 HIV Attacks the Immune System via the CD4$^+$T-cell

HIV attacks the immune system by targeting the heart of the immune system, the CD4$^+$T-cells which are the conductors of the immune system. When a pathogen is introduced into the body as either a virus or a bacterium, it is first recognized by CD4$^+$T-cells. The CD4$^+$T-cells are responsible for coordinating each of the host immune defences, namely the killer cells, the antibodies and the phagocytes that will eliminate the pathogens (Autran & Katlama, 2000). However, if the CD4$^+$T-cells are destroyed, the immune defences that remain become less functional and are unable to eliminate the pathogens and the latter can proliferate to cause disease (Autran & Katlama, 2000). In the body, HIV is replicating in its niche, which is the memory CD4$^+$T-cells-the cells that are very actively mobilized against the most current infections. Each time a memory CD4$^+$T/cell is infected by HIV and each time HIV is replicating in a memory CD4$^+$T-cell, this cell dies and is eliminated. In the healthy young adult or at the beginning of the HIV infection, these dying memory CD4$^+$T-cells are replaced by a constant source or a reservoir of naïve CD4$^+$T-cells originating from the thymus. It is reported that during the course of HIV infection, about one billion HIV particles are produced per day, resulting in increasing numbers of infected CD4$^+$T-cells. The infection spreads in the memory cells, in the naïve CD4$^+$T-cells and in the thymus; the source is therefore progressively exhausted, surpassing the capacity to produce new CD4$^+$T-cells (Autran, 2000).

Scientists have observed that progressive loss of CD4$^+$T-cells is the cardinal manifestation of the effects of HIV infection. The CD4$^+$T-cell count can therefore serve as a useful indicator of the severity of the infection, providing both a convenient measure of immunological status and giving some indication of the risk of opportunistic infections and neoplasia, particularly in individuals who have already begun to show a substantial CD4$^+$T-cell decline. The CD4$^+$T-cell count indeed has considerable importance in some systems used to stage HIV infection and by implication the guidance of treatment decisions. It is particularly important in determining whether it is appropriate to initiate prophylactic therapy for opportunistic infections (Bagasra *et al.*, 1992; Hughes *et al.*, 1997). The significance of the destruction of CD4$^+$T-cells in an HIV-infected person becomes evident when their functions are considered.

2.9.4.5 CD4$^+$T-cell Depletion in HIV Disease

In many parts of the world, most of the time, infection with the HIV leads to death if not treated. In time, it became clear that the virus could replicate within human CD4$^+$T-cells *in vitro*, that the viral envelope protein could bind to CD4$^+$T-cell and circulating CD4$^+$T-cells decreased in number as the disease progressed (Martin, 2000). It was also apparent that CD4$^+$T-cells were crucial in coordinating cellular and humoral immune responses against exogenous antigens. As a result of these observations, it was not difficult to imagine that HIV-associated immunodeficiency was due to virally mediated destruction of CD4$^+$T-cells (McCune, 2001).

(i) Evidence for CD4$^+$ T-cell Depletion

There is a growing realization th[illegible]cyte compartment is comprised of multiple subpopulations and that there are inadequate means by which to monitor the relative growth, death and movement of these subpopulations in an individual patient over time. The definition of CD4$^+$T-cell loss in HIV disease thus remains unclear (McCune, 2001). Most researchers agree that HIV infection results in the progressive loss of CD4$^+$T-cells from circulation as well as the depletion of CD4$^+$T-cells from total body stores (Haase, 1999; Wong *et al.*, 1997; Martin, 2000). Quantitative estimates, using a variety of assumptions, indicate that the normal young (<30 year old) adult harbours about 2×10^{11} mature CD4$^+$T-cells (Haase, 1999). In the HIV-infected patient, this total number is halved by the time the peripheral blood CD4$^+$T-cell count falls to 200 cells/ml. As the HIV infection progresses to a more advanced stage, destruction of parenchymal lymphoid spaces is so extensive that enumeration of the total body CD4$^+$T-cell count has not been attempted. With further disease progression, there is also a decrease in the proportion of resting naïve (CD45RA$^+$CD62L$^+$) T-cells and an increase in the proportion of activated memory/effector (CD45RO$^+$) T cells; concomitantly, the T-cell receptor (TCR) repertoire is both perturbed and restricted. Among those cells that persist, many may be dysfunctional.

In summary, HIV induces both quantitative and qualitative defects in the CD4$^+$T-cell compartment and the circulating CD4$^+$T-cell count continues to be one of the best surrogate markers by which to gauge prognosis in late infection and to trigger treatment interventions (Haase, 1999; McCune, 2001).

(ii) Possible Causes of CD4$^+$ T-cell Depletion

Several mechanisms have been proposed to explain HIV-mediated depletion of CD4$^+$T-cells. All are based on experimental observations (Martin, 2000; McCune, 2001). The total body CD4$^+$T-cells may be depleted in absolute number because they are destroyed or because their production is impaired. In addition, the fraction of circulating cells may decrease if viral infection results in their redistribution out of the intravascular space and into the confines of lymphoid organs (McCune, 2001; SAHIVCS, 2001).

The balance of destruction an[illegible] ne important factor that can be explained by multiple mechanisms. It is possible, for example, that $CD4^{+}$T-cell depletion is related directly to the virally mediated destruction of infected cells. On the other hand, physiological responses to HIV infection might initiate events that result in the destruction of uninfected cells. In either case, loss of mature cells should be compensated for by increased production of new cells and mature $CD4^{+}$T-cell depletion should occur only if cells lost in the periphery cannot be replaced. The devastating feature of HIV infection is that the virus can have direct and indirect pathogenic effects on both mature $CD4^{+}$T-cells and on the progenitor cells from which they arise (McCune, 2001).

(a) Accelerated Destruction of Mature $CD4^{+}$T-cells

Early experiments done with laboratory-adapted HIV isolates in tissue culture revealed a cytopathic virus with exquisite tropism for $CD4^{+}$T-cells. The provision of potent (protease inhibitor-containing) antiretroviral medications to patients with advanced HIV disease caused the viral load to drop and the $CD4^{+}$T-cell count to rise. By making reasonable and largely accepted assumptions about T-cell distribution and by assuming that antiretroviral therapy does not alter the production rate of T-cells, the statement was interpreted to mean that, before therapy, continuous rounds of infection sustained the viral load and that as many as 2×10^{9} infected $CD4^{+}$T-cells were destroyed per day. By extension, HIV disease is a high state, accelerated destruction of mature $CD4^{+}$T-cells and leads to eventual exhaustion of the immune system (Ho *et al.*, 1995; Wei *et al.*, 1993). In HIV-infected human subjects, quantitative image analysis revealed decreased numbers of $CD4^{+}$T-cells and increased levels of cellular proliferation and apoptosis in lymphoid tissue (Ho *et al.*, 1995; McCune, 2001).

(b) Chronic Activation a[illegible]ath

It is agreed that $CD4^+$T-cell death may occur in uninfected cells as a by-product of HIV infection of other cells. According to Grossman & Herberman (1997), HIV disease is typified by a state of chronic activation driven in part by the antigenic stimulus of HIV and in part by an antigen-independent mechanism. For instance, cytokines are released by apoptotic cells and activated T-cells. Multiple bursts of activated cells spread throughout the body and would be characterized by apoptotic cell-mediated activation of resting lymphocytes, cytokine-driven expansion of responding cells and contraction of the responding population by activation-induced cell death. It is believed that if apoptotic cells are infected by or otherwise carry HIV, antigen-specific cell activation could support virus dissemination to responding $CD4^+$T-cells, irrespective of their TCR specificity (Pope, 1994).

Reciprocally, the virus may be spread upon activation of non-productively infected $CD4^+$ memory T-cells in the context of immune responses to HIV or other antigens. During the asymptomatic phase of infection, when the fraction of infected cells is much lower than the fraction of activated cells, these bursts would predictably continue in a local, recurrent and asynchronous fashion, and $CD4^+$T-cell depletion might be driven by several mechanisms (Bentwich *et al.*, 1999). It has been suggested that the relentless activation of naïve cells into the activated/memory pool may not be fully compensated for by replenishment of new naïve cells from the thymus or by the generation of viable memory cells (Hazenberg *et al.*, 2000). Alternatively, chronic stimulation of resting T-cells might have a negative effect on the homeostatic regeneration of these cells. The relevance of this process to $CD4^+$T-cell depletion is underscored by the observation that disease progression is associated with immune activation and vice versa (Wilson, 1990; Bentwich *et al.*, 1999).

(c) Impaired Production of New $CD4^+$T-cells

This mechanism focuses on mature $CD4^+$T-cells, but these cells are often derived from early progenitors that may also express $CD4^+$T-cells. Such progenitors, including multi-lineage and lineage-restricted haematopoietic progenitor cells are uniquely endowed with the capacity to persist with long half-lives and to generate large numbers of differentiated progeny rapidly upon stimulation. If these cells are destroyed or rendered non-functional, mature progeny could not be made (McCune, 2001). Evidence for suppression of multi-lineage and lineage-specific haematopoiesis has been available since the beginning of the AIDS epidemic (Pakker, 1998).

Laboratory findings showed that when late-stage patients initially presented with opportunistic infections, they were not just lymphopenic, but anaemic, neutropenic and thrombocytopenic as well. These findings led to multiple diagnostic bone-marrow biopsies, the results of which were frequently abnormal. Microscopic examination revealed hypercellularity or hypocellularity, plasmacytosis, myeloid or erythroid dysplasia and a variety of other pathological changes (McCune & Kaneshima, 1995). Phenotypic and functional analysis of bone-marrow progenitor cells showed a decrease in the number of lineage-restricted colony-forming units and in some, but not all instances, infection and/or apoptotic death of CD4$^+$T-cell progenitors. Although the mechanisms associated with such cytopenias remain unclear, they are often reversed upon the provision of effective antiretroviral therapy (Fleury, 1998).

The thymus, housing many CD4$^+$T-cells in varying stages of maturation, is another critical target organ for HIV infection. Examination of paediatric and adult specimens has revealed thymocyte depletion, loss of corticomedullary demarcation and development of thymic medullary B-cell follicles. These changes are associated with immunohistochemical visualization of structural proteins within thymocytes and are evidence of viral replication. Although it has proven difficult to study the thymus in HIV-infected humans, the frequency of circulating CD4$^+$ and CD8$^+$ naïve T-cells have been found to decrease as disease progresses (Roederer, 1995). In addition, cells bearing TCR excision circles also decrease in frequency with age and as a function of HIV disease progression (Zhang, 1998). In return, signs of thymopoiesis return after treatment of some individuals with effective antiretroviral therapy particularly if they are younger and have evidence of plenty thymocytes by computed tomography (Zhang, 1998). It has also been noticed that peripheral lymphoid organs undergo marked alterations after HIV infection. These changes include the accumulation of virus on and eventual destruction of the follicular dendritic cell network, decompartmentalisation and depletion of both the $CD4^+$ and $CD8^+$T- cell populations. Thus, HIV infection leads to profound disruption of the bone marrow, thymus and peripheral lymphoid organs and, where measurable, quantitative and qualitative defects in important $CD4^+$T progenitor cells (Roederer, 1995). With these cells eliminated or no longer functional, the immune system cannot be sustained.

2.9.4.5 $CD4^+$T-cell Count and Prognosis

The $CD4^+$T-cell count has been shown in numerous clinical trials and natural history studies to be an independent risk factor for progression to AIDS and death. However, it has two major limitations: it is subject to considerable variation, for instance, inter-assay variability, diurnal variation, changes due to inter-current illness and thus it reflects existing damage to the immune system (Mellors *et al.*, 1995).

2.9.5 Significance of Viral Load

Research studies have demonstrated that persons with symptomatic HIV infection or AIDS have significantly a higher titres of plasma HIV RNA than those with asymptomatic infection. It is also true from these studies that those with a high viral load have greater risk of disease progression than those with a lower viral load (Metcalf *et al.,* 1997). It has been estimated that individuals who have plasma HIV RNA levels > 100 000 copies/ml within six months of sero-conversion are 10 times more likely to progress to AIDS within 5 years than those with plasma HIV RNA levels <100 000 copies/ml (Saag *et al.*, 1996). In one study, the median survival time among individuals with baseline plasma HIV RNA levels >36 270 copies/ml was 5.3 years, and that among individuals with plasma HIV RNA levels < 4 500 copies/ml was >10 years (Hughes *et al.*, 1997).

2.10 Nutrition and the Immune System

2.10.1 Introduction

It is generally accepted that nutrition is an important determinant of immune responses (Chandra, 1997). Results from epidemiological and clinical studies suggest that nutritional deficiencies alter immuno-competence and increase the risk of infection. It is agreed that poor sanitation and personal hygiene, overcrowding, contaminated food, water and inadequate knowledge of nutrition contribute to susceptibility to infection. Previous research findings have confirmed that impaired immunity is a critical adjunct factor in malnutrition-associated infection. This concept does not only apply to people in developing countries but to people (all age groups) in all countries (Chandra, 1991; Chandra, 1996; Chandra, 1997; Bell *et al.*, 1997; Bogden *et al.*, 2000).

Adherence of bacteria to epithelial cells is an essential first step before invasion and infection can take place. The number of bacteria adhering to respiratory epithelial cells has been shown to increase in malnutrition as documented by Chandra (1997).

2.10.2 Role of Micronutrients in the Immune System

Several trace elements and vitamins have essential roles in metabolic pathways and immune cell functions. The deficiencies of these micronutrients have been noted and are known to complicate malnutrition and other systemic diseases. Likewise, human malnutrition is usually a composite syndrome of multiple nutrient deficiencies (Bendich & Chandra, 1990; Baum & Shor-Posner, 1998; Dannhauser *et al.,* 1999; Bogden *et al.,* 2000). Five general concepts have been advanced: (1) Alterations in immune responses occur early in the course of reduction in micronutrient intake. (2) The extent of immunological impairment depends on the type of nutrient involved, its interactions with other essential nutrients, the severity of the deficiency, the presence of concomitant infection and the age of the person concerned. (3) Immunological abnormalities predict outcome, particularly the risk of infection and mortality. (4) For many micronutrients, excessive intake is associated with impaired immune responses. (5) Tests of immuno-competence are useful in titration of physiological needs and in assessment of safe lower and upper limits of micronutrient intake (Chandra, 1997; Dannhauser *et al.,* 1999). An established effect of nutrition on immunity has led to several practical applications and its usefulness is still relevant today.

2.10.3 Nutrition and HIV Infection

The relationship between nutrition and HIV/AIDS is well recognized. In fact, in Africa AIDS was initially known as "Slim Disease" because of the classical wasting typically experienced by persons with the disease (Piwoz & Preble, 2000). HIV infection compromises the nutritional status of infected persons and in turn poor nutritional status can affect the progression of HIV infection (Friis & Michaelsen, 1998; Niyongabo *et al.*, 1999; Piwoz & Preble, 2000; Fawzi, 2003).

From a clinical view, infections may affect the nutritional status of an individual suffering from HIV/AIDS in various ways, such as a reduction in food intake and nutrient absorption and by increasing the utilization and excretion of proteins and micronutrients (Semba & Tang, 1999). It has also been observed that HIV infection accelerates the release of pro-oxidants, cytokines and other reactive oxygen species, leading to the increased utilization of antioxidants such as vitamin E, C, beta-carotene and micronutrients such as iron, zinc, selenium, manganese and copper (Friis & Michaelsen, 1998). An imbalance between these pro-oxidants and antioxidants causes oxidative stress which further damages the cells, proteins and enzymes, thus accelerating HIV replication (Schwartz, 1996).

It has been shown that deficiencies of nutrients may affect the immune function in ways that may influence viral expression and replication, which further affect progression of HIV disease and mortality of the patient (Semba & Tang, 1999). Hormones such as glucagons, insulin, epinephrine and cortisol which are involved in the metabolism of protein, carbohydrate and fat, have been reported to be affected by HIV infection (Macallan, 1999). Increased levels of these hormones is believed to contribute to weight loss and the wasting syndrome seen in HIV/AIDS patients (Macallan, 1999; Piwoz & Preble, 2000). Research studies have confirmed that nutrient deficiencies are associated with immune dysfunction and accelerated progression to AIDS (Fawzi & Hunter, 1998; Macallan, 1999). Furthermore, deficiencies of protein and essential fatty acids interfere with immune function.

2.10.3.1 Diet and Nutrition in Africa: Current Status

Certain foods are preferentially utilized in specific African countries and communities (Watson, 1994). The important staple foods eaten by Africans and the protein-energy percentages of these staples are: wheat (11%), millet (12%), rice (8%), maize (10%), yam (6%), plantain (4%) and cassava (3%) (Watson, 1994). The major meals are bulky and the amount of staple food usually consumed per average person per day does not provide the recommended daily allowance (RDA) of energy, protein and other essential nutrients (Hiel *et al.*, 1982; Enwonwu, 1992; Moore *et al.*, 1997 & 1998).

These traditional foods are often prepared and served under poor hygienic conditions, thus setting the stage for repeated episodes of diarrhoea, thus aggravating the poor nutritional status (Abrams *et al.*, 1993; Anyanwu & Enwonwu, 1995). For most African countries, the first decade of the HIV/AIDS epidemic was a period of severe economic crisis characterized by an unsustainable foreign debt burden, monetary devaluation and ten-to twenty-fold rise in consumer price indices. Reports have linked malnutrition with HIV infection and AIDS, hence it is appropriate to examine malnutrition in Africa and its relationship with HIV infection and AIDS.

2.10.4 Malnutrition

Improving the nutrition situation in Africa has been a challenge for decades, complicated by a combination of individual, household, community, national and international factors, including, in the last two decades, the emergence of HIV infection and AIDS (Castetbon *et al.*, 1997; Piwoz & Preble, 2000). It has been reported that disease, cultural beliefs and customs, high fertility rates, poor economic status and limited access to health and other social services also contribute to chronic, endemic malnutrition on this continent (Ndure *et al.*, 1999). Malnutrition is said to take many forms. These include protein-energy malnutrition (measured in terms of body size) and micronutrient malnutrition, which in its mild and moderate forms is not always recognized and is often referred to as "hidden hunger" (Ndure *et al.*, 1999).

Ndure *et al.* (1999) reported that deficiencies in vitamins and minerals such as vitamin A, B complex, iron and iodine, that are vital for the body's normal function and immune system, occur in populations with high infectious disease burdens. These deficiencies have been associated with poor quality diets characterized by limited consumption of animal products and seasonal or periodic food insecurity. Recent data suggest that little or no progress has been made in reducing the malnutrition in sub-Saharan Africa in the last twenty years (Watson, 1994; Niyongabo *et al.,* 1999; Piwoz & Preble 2000. It is also indicated that, in several countries, malnutrition is increasing as a result of armed conflicts, deteriorating health systems, shrinking economies and HIV/AIDS (Murray & Lopez, 1996).

Malnutrition among African people and especially among women of childbearing age is also a serious problem, with an estimated 42% of African women as a whole and half of the pregnant women suffering from anaemia (ACC/SCN, 2000). Between 10% and 20% of Africans between the ages of 20 to 49 years are underweight and nearly 50% of the African population is at risk of developing micronutrient deficiency diseases (Baker *et al.*, 1996; Mocroft *et al.*, 1999). The consequences of malnutrition include acquisition of some kinds of infections as well as reduced labour productivity (Baker *et al.*, 1996).

2.10.4.1 Pathogenesis of Malnutrition in HIV/AIDS

Food intake is inhibited indirectly in patients with malabsorption, resulting from different diseases or systemic infections due to the release of specific factors that inhibit appetite at the central nervous system level (Wheeler *et al.*, 1998; Kotler *et al.*, 1999). These problems may be exacerbated by economic factors or other impediments to obtaining food (Kotler, 1992).

(i) Localized Pathology and Nutrient Malabsorption

Watson (1994) indicated that oesophageal ulcers of viral, mycobacterial and neoplastic varieties were known to affect food intake. Anorexia may be a side effect of various medications. It is also known that neurological disease may impair appetite or produce swallowing disorders. There is also evidence that unabsorbed micronutrients such as vitamin A, zinc and iron in the lower bowel (ileum and colon) are associated with signals that decrease appetite (Hecker & Kotler, 1990).

Repletion of body cell mass through effective treatment of disease and by proper nutrition is an important consideration, although repletion is difficult, if not impossible, in patients with untreated serious disease complications (Watson, 1994).

(ii) Weight Loss & Lean Body Mass

The importance of this progressive condition has been reinforced by the expansion of the AIDS case definition to "HIV Wasting Syndrome" by the Centers for Disease Control and Prevention (Watson, 1994). Weight loss as well as low serum albumin levels are predictive of an increased risk of morbidity and mortality in hospitalized patients. Studies have established that the frequency of complications from malnutrition increases sharply when serum albumin levels fall below 30 g/l (Trujillo *et al.*, 1992; Babamento *et al.*, 1994; Macallan, 1998).

HIV/AIDS patients typically lose 46% of their potassium by the time of death, and this potassium is lost over the last nine months of life. The patients also lose 34% of their ideal body weight during the last 4 to 5 months prior to death (Babamento *et al.*, 1994).

In a study reported by Watson (1994), lean body mass (estimated from the potassium pool) was greater than the loss in body weight. The amount of lean body mass is sufficient in itself to be the cause of death. World War 2 era starvation studies point out that loss of less than 40% of body weight resulted in death (Watson, 1994). Studies of body composition in AIDS patients demonstrate that body cell mass depletion is out of proportion to losses of body weight or fat and findings indicated that nutritional support can improve the nutritional status of HIV/AIDS patients (Watson, 1994; Piwoz & Preble, 2000).

(iii) Relationship between HIV/AIDS & Malnutrition

The epidemiology of malnutrition and of infectious diseases is intermingled particularly in impoverished communities in the Third World (Maletnlema, 1991; Scrimshaw & SanGiovanni, 1997). The relationship between HIV/AIDS and malnutrition presents a classical example of the well-recognized "vicious cycle" of immune dysfunction, infectious diseases and malnutrition. Changes in the immune function due to malnutrition are strikingly similar to those induced by HIV/AIDS. In fact, for many years, the impairment to immune function caused by malnutrition has been referred to as the "Nutritional Acquired Immune Deficiency Syndrome" or NAIDS (Savage & Burgess, 1995; Piwoz & Preble, 2000). Since normal immune function is dependent on good nutritional status, some researchers have come forward with the hypothesis that malnutrition is the predominant underlying cause for the full clinical expression of AIDS in HIV-seropositive persons (Maletnlema, 1991; Piwoz & Preble, 2000). According to these authors, inadequate nutrition may influence specific systems involved in the progression from asymptomatic HIV infection to the full-blown condition of AIDS, as well as intensify the susceptibility to opportunistic infections, and may also contribute to the severity of HIV-related disease (Kelly *et al.*, 1999). Malnutrition has clinical and social implications and is thus examined below.

(a) Clinical Implication

Long before the AIDS epidemic emerged in Africa in the early 1980s, the synergistic interactions between infections, nutritional status and immune function were recognized. Infectious diseases, no matter how mild, influence nutritional status and conversely, almost any nutrient deficiency, if sufficiently severe, will impair resistance to infection (Scrimshaw & SanGiovanni, 1997). The physical environments contain infectious microbes including viruses, bacteria and fungi. These are often more prevalent in Africa than in industrialized countries. In healthy individuals, the immune system protects the body from damage by these microbes. It has been noted that people with HIV/AIDS, whose immune systems are compromised, have difficulty in resisting a variety of serious infections (Piwoz & Preble, 2000). HIV acts by replicating inside host cells. To eliminate the infection, the immune system must recognize and destroy these infe... ...ells that mediate immunity include lymphocytes. Among the lymphocytes, $CD4^{+}$T-cells (also called T4 cells and T-helper cells) are critical to the immune system functioning. HIV infection destroys $CD4^{+}$T-cells and leads to a deterioration of the overall immune system (Chandra, 1997, Martin 2000; Bouic *et al.*, 2001; Fawzi, 2003).

Infections affect nutritional status by reducing dietary intake and nutrient absorption and by increasing the utilization and excretion of protein and micronutrients as the body mounts its acute phase response to invading pathogens (Maletnlema, 1991; Chandra, 1997). Infections also result in the release of pro-oxidant cytokines and other reactive oxygen species. This leads to the increased utilization of anti-oxidant vitamins, for example vitamins E, C and beta-carotene as well as sequestration of several minerals that are used to form anti-oxidant enzymes (Friis & Michaelsen, 1998).

(b) Social Implication

Malnutrition associated with HIV infection has serious and direct implications for the quality of life of people living with HIV and AIDS (Watson, 1994; Chandra, 1997). Weight loss is often the event that begins a vicious cycle of increased fatigue and decreased physical activity, including the inability to prepare and consume food (Norse, 1991; Babamento & Kotler, 1997; Fawzi & Hunter, 1998).

Malnutrition associated with HIV/AIDS affects entire families and their dependants and they require continuous care during bouts of illness. In parts of Africa where farming is a primary occupation and nutritional requirements are usually met through local food production, HIV/AIDS among agricultural workers is affecting farm incomes, food productivity and nutritional status (Babamento & Kotler, 1997).

2.11 Nutritional Consequences of HIV Infection in HIV/AIDS Patient

To understand the relationship between nutrition and HIV/AIDS, one must consider the effect of the disease on the body size and composition (weight, lean body mass and body cell mass) as well as the effect on the functioning of the immune system.

Nutrition plays a role in each of these conditions. One must also keep in mind that malnutrition may be a contributor to HIV disease progression as well as a consequence of the disease (Baum *et al.,* 1995; Babamento *et al.*, 1994; Beck, 2000; Piwoz & Preble, 2000). In populations in which malnutrition is endemic, body size and composition changes associated with protein-energy malnutrition may always be associated with deficiencies in vitamins and minerals which are important for the functioning of the immune system.

The wasting syndrome typically found in adult AIDS patients in Africa is a severe nutritional manifestation of the disease (Babamento *et al.*, 1994). It has been noted that weight loss typically follows two patterns in people living with HIV/AIDS: slow and progressive weight loss from anorexia and gastrointestinal disturbances, and rapid, episodic weight loss from secondary infections. Observations have also shown that even relatively small losses in weight (5%) have been associated with decreased survival in individuals with AIDS (Boelaert *et al.,* 1996; Castaldo *et al.*, 1996; Macallan, 1999). Research studies have shown that weight loss and wasting in HIV/AIDS patients may develop as a result of three major overlapping processes (Babamento *et al.*, 1994; Keating *et al.*, 1995; Macallan, 1999). These major overlapping processes are discussed below.

2.11.1 Reduction in Food Intake

This may be due to painful sores in the mouth, pharynx and oesophagus. Some data have shown that fatigue and depression, including changes in mental state, may also play a significant role by affecting appetite and interest in food (Babamento *et al.,* 1994). Reductions in food intake are believed to be an important cause of slow and progressive weight loss (Abrams *et al.*, 1993; Macallan, 1999).

2.11.2 Nutrient Malabsorption

Malabsorption that accompanies frequent bouts of diarrhoea due to intestinal parasites and other pathogens has been reported in people with HIV/AIDS. Some HIV-infected individuals have demonstrated increased intestinal permeability and other intestinal defects in early infection (Keating *et al.*, 1995; Macallan, 1999).

Malabsorption of fats and carboh... found to be common at all stages of HIV infection in both adults and children (Semba & Tang, 1999). It has been pointed out that fat malabsorption, in turn, affects the absorption and utilization of fat-soluble vitamins.

2.11.3 Metabolic Alterations

Changes in metabolism occur during HIV infection from severe reduction in food intake as well as from the immune system's response to the infection. When food is restricted, the body responds by altering insulin and glucagon production that regulate the flow of sugar and other nutrients in the body (Cimoch, 1997). Over time, the body uses up its carbohydrate stores from muscle and liver tissue and then begins to break down body protein to produce glucose. This process could cause protein loss and muscle wasting (Grimble, 1990, Babamento & Kotler, 1997; Cimoch, 1997).

Existing studies suggest that infections result in a loss of 0.6 g to 1.2 g of protein per kilogram body weight per day in adults when amino acids are mobilized from skeletal muscle for gluconeogenesis, synthesis of immune proteins and enzymes in response to the release of cytokines (Fawzi & Hunter, 1998). For these reasons, protein requirements are substantially higher in HIV-infected individuals than in non-HIV-infected persons (Fawzi & Hunter, 1998; Scrimshaw & SanGiovanni, 1997).

2.12 Relevance of Nutritional Factors to the Progression of HIV Infection

The host's response to infection and inflammatory stimuli is mediated by cytokines (Haynes *et al.*, 1996; D'Souza & Harden, 1996). Cytokines are a range of polypeptides that have multiple biological activities including enhancing the attraction, proliferation, activation and differentiation of white blood cells, as well as mediating a wide range of metabolic alterations (Grimble, 1990). Cytokine production and actions are affected by malnutrition (Grimble, 1990; Cimoch, 1997). Abnormalities in cytokine production characterize HIV infection. Impaired cellular anti-oxidant status has been identified as a consistent prominent feature of protein-energy malnutrition and other forms of malnutrition. This may play a key role in the rapid replication of

HIV in malnourished individual[illegible]2; Semba *et al.*, 1998; Piwoz & Preble, 2000). It has been demonstrated that in a chronically HIV-infected T lymphocyte cell line, non-toxic concentrations of ascorbate in the cell culture medium reduces the level of extra-cellular reverse transcriptase by 99% and the expression of p24 antigen by 90% (Harakeh *et al.*, 1990).

2.12.1 The Role of Vitamins and Minerals in HIV/AIDS Patients

Many vitamins and minerals (also referred to as micronutrients) are important to the HIV/nutrition relationship due to their critical roles in cellular differentiation, enzymatic processes, immune system reactions and other body functions (Fawzi & Hunter, 1998). The role of micronutrients in other infectious diseases such as measles, diarrhoea and respiratory infections has been extensively studied and it is known that several vitamins and minerals are required by the immune system and major organs to fight infectious pathogens (Fawzi & Hunter, 1998; Semba & Tang, 1999). Persons with inadequate intakes, blood levels or body stores of these micronutrients have difficulty in resisting infection. As a result, the role of micronutrients in HIV/AIDS is of special importance in individuals and populations with marginal or low micronutrient intakes (Friis & Michaelson, 1998). This applies to most AIDS-affected Africans and, as already noted, micronutrient deficiency is endemic (Piwoz & Preble, 2000).

Studies in both industrialized and developing countries have confirmed that HIV-infected individuals have decreased absorption, excessive urinary losses and low blood concentrations of vitamins A, B_1, B_2, B_6, B_{12}, C, E, as well as of folate, beta-carotene, selenium, zinc and magnesium (Tang & Smit, 1998). At present, it is not known whether these deficiencies are independent markers of disease progression resulting from a compromised immune system, or whether they are causally related to the development or exacerbation of symptoms of HIV/AIDS. This distinction is important in order to determine whether nutritional therapy and management can retard or alter the course of the disease.

2.12.2 Micronutrients and HIV Disease Progression and Mortality

Review studies published by Tang & Smit (1998), Fawzi & Hunter (1998), Baum & Shor-Posner (1998) and Semba & Tang (1999), have concluded that micronutrient deficiencies associated with HIV infection vary across populations and according to the disease stage are associated with an accelerated progression of HIV infection to AIDS and are predictive of AIDS related mortality. Micronutrient supplementation, unlike many other AIDS treatments, has the potential in Africa to be an affordable and relatively easy way to deliver a public health measure. The roles of specific micronutrients in HIV disease progression and mortality and findings from published intervention trials are summarized below.

2.12.2.1 Vitamin A

Of all the micronutrients, the role of vitamin A in HIV infection has received the greatest attention in Africa. This is because of its well-known role in affecting child morbidity and mortality as well as early observations that vitamin A status was associated with increased risks of mother-to-child-transmission (MTCT) of HIV (Semba et al., 1994); with HIV viral load in breast-milk and vaginal secretions (Nduati *et al.*, 1995; John *et al.*, 1997); with progression to AIDS (Tang *et al.*, 1993; Tang *et al.*, 1996); with adult survival (Semba *et al.*, 1994, Semba, 1997), and with infant morbidity (Coutsoudis *et al.*, 1995) and mortality (Dushiminana *et al.*, 1992). The potential for vitamin A supplementation to impact positively the course of HIV/AIDS is worth pursuing since vitamin A is beneficial in HIV-negative populations, is inexpensive and is relatively easy to administer with minimal side effects, however megadose can be dangerous to health (Semba *et al.*, 1994; Nduati *et al.*, 1995).

Vitamin A deficiency may be caused by insufficient dietary intake of vitamin A-rich food, malabsorption, impaired storage (because of liver disease), and/or increased utilisation or urinary loss of vitamin A during acute and chronic infection (Semba, 1997). Vitamin A deficiency causes growth retardation and xeropthalmia, and increases the incidence and/or severity of many infections. While vitamin A deficiency is relatively rare among HIV-negative adults in industrialized countries, up to one-third of HIV-positive adults in industrialized countries may be vitamin A deficient (Kennedy *et al*., 2000). The percentage incidence of vitamin A deficiency in HIV-positive people in developing countries, including Africa, is reported to be higher (Nimmagadda *et al*., 1998). Vitamin A forms a key ingredient in the liquid supplement under investigation in this study.

2.12.2.2 Vitamin E

Vitamin E is necessary for the proper functioning of the immune system and it increases humoral and cell-mediated immune responses, including antibody production, phagocytic and lymphocytic responses and resistance to viral and infectious diseases (Odeleye and Watson, 1991; Watson, 1994). The oxidative stress created by HIV and related opportunistic infections increase the utilization of antioxidant vitamin E, possibly leading to deficiency. Vitamin E deficiency, in turn, further debilitates the immune system because of its role in immune stimulation and functioning, leaving people with HIV/AIDS more susceptible to opportunistic infections (Piwoz & Preble, 2000).

Studies in the USA found that high baseline serum vitamin E levels were associated with decreased HIV progression after taking into account HIV-related symptoms, $CD4^{+}T$ cell count, age and other confounding variables (Tang *et al*., 1997). Individuals with serum vitamin E levels greater than or equal to 23.5 mmol/l took 34% longer time to develop AIDS compared with those with low serum vitamin E levels (Tang *et al*., 1997). Another study in Canada found that three months of supplementation with vitamin E (800 IU) and vitamin C (1000 mg) significantly reduced oxidative stress and HIV viral load (Allard *et al*., 1998). A study in Zambia among AIDS patients suffering from persistent diarrhea found that vitamin E deficiency at enrolment predicted mortality in the following month (Kelly *et al*., 1999). Vitamin E is also included in the liquid supplement used to investigate its influence on the immune status of HIV-positive/AIDS patients.

2.12.2.3 Vitamin C

Research studies have suggested that at high doses (greater than 1000 mg), vitamin C has a unique pharmacological function, displaying the potential to serve as an antioxidant and primary source of electrons under conditions of drug-induced glutathione (GSH) deficiency or severe free-radical toxicity (Cathcart, 1991; Martenson & Meister, 1991; Semba & Tang, 1999. Due to the fact that antioxidant depletion and a chronic scorbutic-like state are associated with HIV/AIDS, metabolic functions of vitamin C essential for preventing these conditions in healthy subjects are potentially relevant to the control and management of such conditions in persons with HIV infection and AIDS (Watson, 1994).

HIV/AIDS patients manifest striking GSH deficiencies and often exhibit symptoms of acute-induced scurvy characterized by life-threatening weight loss, brittle bones and swollen glands (Cathcart, 1991). It has been reported that GSH deficiency can result from changes in dietary vitamin C intake. Volunteers fed controlled diets containing vitamin C at levels lower than the recommended daily allowance (60 mg/dl) had decreased concentrations of GSH in blood plasma (Watson, 1994). Administration of an ascorbate repletion diet (250 mg/dl) in the same study resulted in restoration of the plasma GSH level (Martensson & Meister, 1991; Watson, 1994). It has been pointed out that at high concentrations (obtained through supplementation), vitamin C can act directly to scavenge free radicals as well as convert oxidized forms of non-enzymatic scavengers (tocopherol and GSH disulphide) to their reduced states. Under these conditions, vitamin C functions as a direct source of high energy electrons, saves GSH and acts as an essential antioxidant in the presence of GSH deficiency (Watson, 1994; Martensson & Meister, 1991). Cycles of reactive oxygen intermediate production in HIV-infected persons may induce them to consume free-radical scavengers at an increased rate, leading to the depletion of vital antioxidants in the body.

(i) Vitamin C: Immune Function and Antibacterial Effects

Vitamin C has been found to affect immune function in several ways. It can stimulate the production of interferons, the proteins that protect cells against viral attack. It can stimulate the positive chemotactic and proliferative responses of neutrophils. It has also been shown that vitamin C can stimulate the synthesis of the humoral thymus factor and antibodies of the IgG and IgM classes (Flodin, 1988). Vitamin C deprivation reduces overall complement activity as adequate vitamin C has been found to play an important role in stimulating the C1q biosynthesis. Watson (1994) showed that vitamin C was effective in the inactivation of a wide range of pathogenic bacteria including *Staphylococcus aureus*, *Escherichia coli* and haemolytic *Streptoccoccus* species.

(ii) Antiviral Action of Vitamin C

A striking property of vitamin C is its ability to inactivate viruses and inhibit viral growth in their host cells. Vitamin C has been shown to suppress the human retrovirus expression in immortalized and transformed lymphocytic cell lines (Watson, 1994). It has been further proved that vitamin C is capable of inhibiting HIV replication in both chronically and acutely infected T cell lines in the absence of inducing agents, indicating that the compound (vitamin C) can directly interfere with specific steps in retrovirus replication in differentiated lymphocytic cells (Watson, 1994).

2.12.2.4 Vitamin B_{12}

Vitamin B_{12} deficiency is relatively uncommon in healthy, non-vegetarian populations. However, many studies of people with HIV in the USA reported low serum B_{12} levels even among asymptomatic persons. Low serum B_{12} levels are associated with neurological abnormalities, for example, neuropathy, myelopathy; impaired cognition; reduced $CD4^{+}$T-cell counts, increased bone marrow toxicity and increased mortality (Tang & Smit, 1998). A 9-year study among homosexual and bisexual men with HIV/AIDS in the USA found that men with low serum B_{12} at enrolment (<120 pmol/l) had significantly shorter AIDS-free survival times than men with adequate B_{12} (Tang *et al.*, 1997). Another study of USA men found that improvements in B_{12} levels were associated with increases in $CD4^{+}$T cell count. No studies of vitamin B_{12} and HIV/AIDS in Africa have been identified (Baum *et al.*, 1995).

2.12.2.5 Folic Acid (folate)

Folic acid works closely with vitamin B_{12}, but its role in HIV/AIDS remains unclear. Folic acid is required for the enzymes that produce DNA for replicating and growing cells, including those of the gastrointestinal (GI) tract, blood and growing foetus. Deficiency results in impaired cell division and protein synthesis, causing megaloblastic anaemia. If the GI tract is damaged as is common in HIV-related diarrhoea, folic acid reabsorption may be impaired, setting off a cycle in which deficiency results in further GI tract deterioration and malabsorption of other nutrients (Piwoz & Preble, 2000).

2.12.3 Nutritional Support for HIV-positive/AIDS Patients

As already pointed out, the nutritional status of HIV-positive/AIDS patients is frequently compromised. There is documented evidence that malnutrition will occur at some point in the disease process for more than 95% of patients (Levy, 1989; Fawzi & Hunter 1998). About 65% will experience malabsorption; 95% will have significant weight loss, while about 90% will have oral or oesophageal infections. This may affect food intake (Levy, 1989). Molina (1989) reported a significant reduction in the life-span of HIV-infected patients who had a serum albumin level below 2.5 mg/dl, whereas a serum albumin level of greater than 3.0 mg/dl has been associated with prolonged life span and decreased morbidity. Although data to date, especially in Africa, are limited in this aspect, retrospective data support the role of improved nutritional status and prolonged life with reduced morbidity.

Many HIV-infected patients have demonstrated improved quality of life (increased activity, increased ability to perform activities of daily living, prolonged employment) after adequate provision of nutritional support (Rosenburg & Fauci, 1990). The corner-stone of nutritional support is the nutritional status assessment. The impact of nutritional interventions usually depends on the underlying nutritional status of the individual concerned. If a nutritional supplement is given to correct a deficiency, it is more likely to have an impact than when it is given to persons who are nutritionally replete (Watson, 1994).

2.12.3.1 Nutritional Interventions in Early HIV Disease

The Physicians Association for AIDS Care (1992) and Task Force on nutritional support in AIDS (1989), have established guidelines for nutritional support in early HIV infection which focus on improving oral intake by means of nutrient-dense food supplements and vitamin supplementation. It is suggested that counselling on nutrient selection and food preparation should begin and be periodically reinforced. This, it is believed, will establish a strong link between nutrition and HIV infection in the mind of the patient and health personnel that will alert both parties to more aggressive nutritional intervention when needed later. An optimal strategy would include measurements of energy expenditure and body composition to further the awareness of nutritional changes (Watson, 1994).

2.12.3.2 Nutritional Interventions in Latent HIV Disease

During this stage of the illness, HIV is deceptively dormant in lymphoid and macrophage reservoirs (Pantaleo *et al.*, 1993). However, evidence of ongoing viral replication and immune dysfunction is abundant, as follicular dendritic cells trap HIV particles and follicular lymph hyperplasia proceeds with subsequent activation of germinal B-cells and elaboration of interferon alpha (Pantaleo *et al.*, 1993).

It is documented that $CD4^{+}$ T-cell levels fall at this stage by apoptosis and more rapid progression may occur by syncytia formation (Pantaleo *et al.*, 1993). At this stage the Nutritional Task Force on AIDS (1992) and the Physicians Association for AIDS Care (1989) have established that enteral feeding supplementation be initiated with an intact formula. Although this present study does not focus directly on enteral feeding, it does agree with other authors on the importance of nutrition in the management of HIV infection. If augmented intake cannot be achieved or if weight loss continues despite enteral supplementation, assessment of the mucosal absorptive function must be undertaken. Efforts to stimulate the appetite with mesgesterol acetate or other stimulating agents have led to an increase mainly of fat weight. Again, a multiple strategy may be needed at this stage that provides adequate nutrients along with pharmacological interventions aimed at modifying the metabolic abnormalities driven by cytokines (Vonroenn *et al.*, 1998).

2.12.3.3 Nutritional Interventi… sease

As viral replication continues in lymphoid and macrophage reservoirs, destruction of the follicular dendritic cells in the germinal center occurs, leading to lymphocyte depletion and massive viraemia as the antigen presentation FDC cells degenerate in an accelerated mode related to a lack of cytotoxic activity (Pantaleo *et al.*, 1993). It is known that cytokine activity continues but is propelled by the appearance of opportunistic pathogens. Opportunistic or secondary infections abound and account for most of the digestive and nutritional malfunction (Lahdevirta *et al.*, 1998). It is not until this very advanced stage that too many clinicians finally appreciate that there are nutritional problems with the patients. Body weight falls below its usual pre-illness weight by 20% and lean body mass simultaneously loses proportionately by another 10-15% (Kotler *et al.*, 1989). Kotler *et al.* (1989) have established that death in these patients occurs from malnutrition. Although the Kotler *et al.* (1989) observation is subjective, it does point to the fact that malnutrition plays a significant role in HIV disease progression and death of the patients.

2.12.4 The Importance of Micronutrient Supplementation in Improving the Nutritional and Immunological Status of People Living with HIV/AIDS

Few people, whether or not they are nutritional professionals, would dispute the fact that malnutrition constrains people's ability to fulfil their potential. The joint FAO/WHO Food Standards Programme and the Codex Alimentarius Commission (CAC) were established in 1962 in response to worldwide recognition of the need to ensure the quality and safety of the world's food supply. The effect of HIV on the nutrition of a person follows a number of paths. Health and nutrition depends on the stage and the severity of the infection (Woznicki & D'Alessandro, 1997; Oguntibeju et *al.*, 2003a). It is known that good nutrition in terms of quality and safety can contribute to the well-being of people living with HIV/AIDS at all stages of the disease and may even prolong life (Watson, 1994; Piwoz & Preble, 2000). As a result of malabsorption, the blood micronutrient levels of people living with HIV/AIDS are often lower than those without the infection or syndrome. However, determining the micronutrient status of these subjects is difficult, since infection causes shifting of some nutrients between blood and tissue fluid; consequently micronutrient deficiencies can be caused by malabsorption in addition to poor dietary intake or increased nutritional demand (Connolly *et al.*, 1998).

The aims of nutritional intervention in HIV/AIDS patients are: (a) to minimize loss of lean body mass; (b) to prevent vitamin and mineral deficiencies; (c) to surmount obstacles to nutrient intake and absorption, and (d) to prevent or moderate the use of nutritional approaches that may not enhance the well-being of the patients (Woznicki & D'Alessandro, 1997). It is important for healthcare professionals caring for HIV-infected individuals to understand the relationship between nutrition, HIV infection and the immune system. Many clinicians support the use of dietary supplements by people living with HIV/AIDS (Baum, 1992; Abrams *et al.*, 1993; Fawzi & Hunter, 1998; Oguntibeju *et al.*, 2003b).

It is observed that during the active phases of HIV infection, subjects lose weight and lean body mass rapidly. The probability of imminent death is high when their weight falls to 66% of the actual weight or when lean body mass falls to 54%. Although good nutrition cannot single-handedly stop wasting, nutritional supplementation can reverse immune dysfunction related to malnutrition (Wozninki & D'Alessandro, 1997).

2.12.4.1 Importance of Supplementation in HIV/AIDS Patients

The provision of sufficient food and nutrition to meet people's basic 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/AIDS. 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 has shown that in the early period of HIV infection, weight gain or maintenance might be achieved through nutrition and has helped to reduce the consequences of wasting in people living with HIV/AIDS (Friis & Michaelsen, 1998; van Staden *et al.*, 1998).

With HIV/AIDS, being a disease of the immune system, new strategies, including specific dietary nutrients to improve immune functions, quality of life and prolong survival in infected individuals, could provide additional or alternative approaches for therapeutic treatments in HIV infe... This strategy could also be used in establishing immunity in healthy uninfected persons (Fawzi & Hunter, 1998).

Studies have shown that even people who eat good food are likely to have vitamin and mineral deficiencies when infected with HIV (Cimoch, 1997). For instance, zinc, selenium, magnesium, carotenoids, vitamins A, E, C, B_2, B_6 and B_{12} have all been found to be low in HIV-infected subjects. This can happen before visible sickness and the development of AIDS (Woznicki & D'Alessandro, 1997). The recommended daily allowance of vitamins and minerals is most often not sufficient for people living with HIV/AIDS. Supplementation has been shown to slow disease progression significantly (Semba & Tang, 1999).

In the developing world, in which the majority of the people cannot afford antiretroviral therapy, good nutrition, combined with minerals and vitamins form a good source of therapy. From the mid 1980s until 1990, multiple deficiencies of vitamins and minerals were documented in people living with HIV/AIDS (Macallan, 1999). Although at this time no aggressive supplementation was recommended, a report by FDA, USA (1995), suggested that supplementation of vitamins and trace minerals once or twice the recommended daily allowance (RDA) might offset possible deficits and contribute to meeting increased requirements during hypermetabolic states. In addition, Dwyer *et al* (1998) reported that multivitamin supplements might be helpful for debilitated AIDS patients who suffer from malabsorption. Malnutrition is almost universal among people living with HIV/AIDS, hospitalized or not, largely because of AIDS-related malabsorption. Malnutrition favours opportunistic infections and contributes to wasting (Cimoch, 1997; Macallan, 1999), making supplementation an important aspect in the management of people living with HIV/AIDS.

There have been an increasing number of reports documenting multiple micronutrient deficiencies in HIV-infected/AIDS persons in the absence of proper and adequate supplementation. There have also been increasing recommendations for supplementation in this group of people (Watson, 1994; Macallan, 1999). In pursuance of this goal, members of the Physicians Association for AIDS Care have repeatedly emphasized the importance of supplementation. They carried their concern and objectives with emphasis to the third international symposium on nutrition and HIV/AIDS where they reiterated the importance of supplementation for people living with HIV/AIDS. Research has indicated that HIV-related dementia may be prevented or controlled through good nutrition. Observations from published research studies suggest that many of the negative effects of protein calorie malnutrition (PCM) may be directly or indirectly related to a deficiency of trace elements (Piwoz & Preble, 2000). Baum (1992) recommended supplementation with vitamins A, C, E, the B vitamins, and minerals such as zinc, magnesium and selenium. It was also at about this time that further research documenting the benefits of supplementation became available.

Abrams *et al.* (1993) published the results from two-six year epidemiological studies, one of which was a study of 296 men: a prospective study of dietary intake and AIDS in homosexual men. The authors observed that higher intake of all eleven micronutrients investigated was associated with a higher $CD4^{+}$T-cell count; daily use of multivitamin was associated with a reduced risk of AIDS and a significantly reduced risk of a low $CD4^{+}$T-cell count. In addition, there is increasing evidence that micronutrient supplementation is associated with the absence of, or reduced deficiencies and promotes clinical stability.

Nutritional intervention to prevent or reverse weight loss and wasting with HIV infection may help to preserve independence, improve quality of life and prolong survival. Micronutrient intervention may help to strengthen the immune system and reduce the severity and impact of opportunistic infections in people living with HIV/AIDS. Some nutritional imbalances may directly affect HIV viral replication. Correcting these imbalances may also help to slow HIV disease progression and prolong survival.

More research is essential to determine the dosage of various micronutrients for use by HIV/AIDS patients. Likewise, more defined roles of nutritional supplementation in immune response and in the management of HIV/AIDS patients need to be examined. The above statements partly explain the role that the outcome of this study will play in terms of providing knowledge and the importance of supplementation in HIV/AIDS individuals, especially in the less affluent economic sector of the community.

2.13 Dietary Intake and Weight Loss Patterns in HIV Infection

Wasting and weight loss were recognized early in the AIDS pandemic as frequent and severe complications of HIV infection. Appreciation of the importance of wasting was demonstrated by its inclusion as an AIDS-defining diagnosis in the Center for Disease Control criteria for progression to AIDS (CDC, 1987).

2.13.1 Weight Loss

Weight loss is very common during the course of HIV infection. It is said to occur in up to 50% of individuals at some stage in their illness (Kotler *et al.*, 1989). The impact of wasting on quality of life and self-perception is readily evident to clinicians working with HIV-infected individuals. More objective data can be found in studies which have demonstrated a strong link between loss of body cell mass and reduced survival time, either in terms of time of death or in terms of Kaplan-Meier analysis of survival (Kotler *et al.*, 1989; Suttmann *et al.*, 1995).

Observation by clinical personnel at a community level revealed the impact of wasting, survival and morbidity in HIV-infected patients published by the Community Program for Clinical Research on AIDS. The organization showed that a weight loss trend over a 4-month observation period predicted subsequent mortality; even weight loss trends of only 0-3% over 4 months were predictive of increased mortality (Wheeler *et al*, 1998). Such studies demonstrate at a community level the impact of wasting, reflected by loss of weight which has been demonstrated previously using more complex body composition indices such as body cell mass. This may be partly due to the occurrence of chronic gastrointestinal infections but can also be largely attributed to the frequency of co-infection with tuberculosis in such populations (Serwadda *et al.*, 1985; Lucas *et al.*, 1994; Dannhauser *et al.*, 1999).

2.13.2 Patterns of Weight Change

Research studies have shown that, early in the AIDS pandemic, weight loss tends to be episodic and occurs in association with opportunistic infections (Macallan *et al.*, 1993). Particularly striking was the observation that, following successful treatment of episodes of secondary infecti weight recovery occurred. Such weight gains could be very dramatic, showing that HIV *per se* does not prevent an anabolic response given adequate food intake in an appropriate metabolic environment. It was also noted that some individuals' weight remained stable for prolonged periods despite demonstrable metabolic abnormalities (Kotler *et al.*, 1989). A comparison of weight change patterns showed that the majority of the acute weight loss episodes occurred in relation to opportunistic infections, particularly those that interfered directly with food intake. By contrast, those individuals with a chronic progressive pattern of weight loss often had gastrointestinal diseases (Macallan *et al* 1993; Suttman *et al.*, 1995).

2.13.3 Weight Loss and Energy Balance: Energy Requirements

One of the characteristics of HIV infection appears to be its major impact on metabolic processes. Several studies have investigated metabolic rate in HIV infection. The results have been variable, reflecting the clinical and metabolic heterogeneity of HIV infection, but consensus seems to have emerged that in asymptomatic HIV infection, there is an increase in resting energy expenditure (REE) of about 10% (Schwenk *et al.*, 1996). The heterogeneity of metabolic findings in groups of HIV-infected individuals is particularly striking and makes generalization difficult (Schwenk *et al.*, 1996). Some individuals are considerably hypermetabolic, while others are frankly hypometabolic (Schwenk *et al.*, 1996; Wheeler *et al.*, 1998). In studies including individuals with reduced food intake, the expected compensatory reduction in REE has not been observed and this may represent one aspect of metabolic dysregulation (Schwenk *et al.*, 1996). The presence of secondary infections tends to increase REE further; increases in REE to 29% and 34% above control values were seen in two studies including subjects with secondary infections (Grunfeld *et al.*, 1992). Whether such increases are reflected in total energy expenditure (TEE), the true determinant of energy balance remains less clear. It is difficult to predict the energy requirements for an individual with HIV infection. Even if facilities exist to measure REE, considerable uncertainty still exists. Weight trend is probably the best indicator of adequate energy supply over time (Grunfeld *et al.*, 1992).

2.13.4 Energy Intake and Weight Loss

As mentioned above, weight loss frequently occurs in relation to acute opportunistic infections (Macallan *et al.*, 1993). During such periods, food intake falls markedly (Grunfeld *et al.*, 1992; Ott *et al.*, 1993). In studies performed among HIV positive patients in the United Kingdom, TEE and energy intake were measured during periods of weight stability, gain and loss. It was observed that TEE tended to be lower during weight loss and higher during weight gain phases; changes in TEE could not therefore account for the changes in energy balance necessary to induce such changes in weight. It could be inferred, therefore, that changes in energy intake were the prime movers in determining weight loss. Direct measurement of food intake using a 7-day weighed food intake measurement confirmed this supposition (Macallan *et al.*, 1995). Grunfeld *et al.* (1992) observed that energy intake was similar in HIV-negative controls, asymptomatic HIV-positive subjects and AIDS patients without secondary infection, but was reduced by 36% in AIDS patients with secondary infections in whom intake fell 17% below their REE. Research studies have shown that most HIV-positive individuals do not lose weight because their metabolic requirements are excessive. In fact, the contrary is true: they lose weight because their intake has fallen, often to exceedingly inadequate levels (Grunfeld *et al.*, 1992; Schwenk *et al.*, 1996; Wheeler *et al.*, 1998).

2.14 Body Composition Changes during HIV Infection

2.14.1 Introduction

The relationships among chronic disease, malnutrition and physical debilitation have long been recognized. Clinical and experimental studies have shown that effective treatment of malnutrition may have observable benefits, irrespective of the underlying diseases. This has led to renewed interest in applying nutritional tools in clinical medicine (CDC, 1987).

Malnutrition is a common complication of HIV infection and plays a significant and independent role in its morbidity and mortality. Unexplained weight loss of greater than 10% was established as a clinical criterion for the diagnosis of AIDS by the

Centers for Disease Control and Prevention (CDC, 1987). Unexplained weight loss was one of the most common initial AIDS-defining diagnoses to be reported to public health authorities (CDC, 1987).

Research findings have noted that macronutrients reflect only one aspect of nutritional status (CDC, 1987; Paton *et al.*, 1996; Connolly, *et al.*, 1998). Micronutrient deficiency may exist without macronutrient deficiency, while macronutrient deficiency almost always has associated micronutrient deficiencies (Zangerle *et al.*, 1993; Thea *et al.*, 1995). The concept of malnutrition also has static and dynamic features such as chronic stable malnutrition versus progressive tissue depletion. It is possible for someone to be undergoing a period of weight loss, yet have a nutritional status within the normal range (Kotler, 1992; Paton *et al.*, 1996).

2.14.2 Patterns of Malnutrition in Relation to Body Composition

Starvation and cachexia are the two major patterns of wasting. Starvation can be defined as deprivation of food, either voluntarily or involuntarily, that leads to weight loss. The effects of starvation can be reversed by providing food (Thea *et al.*, 1995; Raghaven *et al.*, 1996). Cachexia is characterized by a disproportionate loss of protein which results from specific alterations in intermediary metabolism (Grundfeld & Feingold, 1992; Macallan, 1999). These metabolic alterations are an integral part of the body's defence, as they provide the energy and substrate needed to fuel the acute-phase response which includes the production of large quantities of specific proteins needed for the clearance of pathogens and tissue debris, and they also damp the oxidative reactions that result from an inflammatory response (Flores *et al.*, 1989; Kelly *et al.*, 1999). The process is regulated by the same cytokines that promote immune responses. In addition, since the metabolic changes are promoted by specific alterations in cellular enzymes, simple feeding may not alter the process substantially. Thus, an important characteristic of cachexia is that depletion of lean tissue may not simply be reversed by feeding the patients (Kotler, 1992; Babamento & Kotler, 1997).

2.14.3 Effects of HIV Infection and AIDS on Body Composition

The earliest studies of nutritional status in AIDS patients conducted between 1981 and 1983 were undertaken with hospitalized patients. Weight loss to an average of about 80% of ideal weight was found. Evidence of protein deficiency was documented by showing deficiencies in serum proteins (transferrin, albumin), haemoglobin and by muscle wasting (mid-arm circumference) (Kotler *et al.*, 1984; Tang *et al.*, 1993; Stack *et al.*, 1996; Mocroft *et al.*, 1999). Several other studies also documented a very high prevalence of severe weight loss in AIDS patients at the time of hospital admission (Serwadda *et al.*, 1985; Niyongabo *et al.*, 1999).

2.15 Wasting Syndrome in HIV/AIDS

2.15.1 Introduction

The wasting syndrome is one of the most pernicious and poorly understood complications associated with HIV infection (Macallan, 1999). In the early days of the epidemic, anecdotal reports of a wasting syndrome associated with late stages of HIV infection began to surface. Although transient weight loss associated with opportunistic infections was also seen, late HIV wasting was often severe, relentless and difficult to treat. This wasting syndrome has become more clearly characterized and is recognized as one of the hallmarks of progressive, untreated acquired immunodeficiency syndrome (Kotler *et al.*, 1985; 1989; Suttmann *et al.*, 1995; Enggelson *et al.*, 1997; Macallan, 1998 & 1999).

In Africa, 60% of AIDS patients presented with wasting (Serwadda *et al.*, 1985; Piwoz & Preble, 2000). In 1987, the Centers for Disease Control (CDC) added wasting syndrome to the list of AIDS-defining illnesses (CDC, 1987). The CDC defined the HIV wasting syndrome as prolonged involuntary weight loss greater than 10% of baseline body weight. The definition also includes conditions such as chronic diarrhoea (at least two loose stools per day for ≥30 days) or chronic weakness and documented fever (≥30 days, intermittent or constant) in the absence of a concurrent illness or condition other than HIV infection that could explain the findings (for example cancer, tuberculosis, cryptosporidiosis or other specific enteritis).

2.15.2 Clinical Studies of HIV-related Wasting

The earliest studies of HIV-associated wasting were clinic-based and focused on AIDS patients presenting with unexplained severe weight loss. Accompanying symptoms affecting the patients' condition and quality of life included fatigue, weakness, anorexia and diarrhoea. An early clue to the importance of opportunistic infections was noted by Kotler *et al.* (1989) who observed that AIDS patients treated for disseminated cytomegalovirus infections with antiviral therapy gained weight, replenished body cell mass and body fat. This shows that wasting at least, when the underlying cause was a treatable infection, was a potentially reversible process.

Severe and progressive decrease in lean body mass has been found to account for the bulk of weight loss in AIDS patients (Kotler *et al.*, 1984; 1989; Zangerlie *et al.*, 1993; Suttmann *et al.*, 1995). It is observed that wasting occurred in the presence and in the absence of diarrhea and the timing of death seemed to correlate with the extent of body-cell-mass depletion rather than the process underlying the loss (Kotler *et al.*, 1985; 1989; 1999). Kotler *et al.* (1989) concluded that death from wasting occurred when body cell mass reached 54% of normal body cell mass and body weight 66% of ideal weight.

Clinical studies have suggested that several mechanisms may be associated with the aetiology of HIV wasting, including enteric pathogens, small intestinal injury and inflammatory changes in the gastrointestinal system. Nutrient malabsorption was documented early on in HIV-infected individuals and, interestingly enough, it was identified in AIDS patients both with and without diarrhoea (Dworkin *et al.*, 1985 and 1990; Smith *et al.*, 1988; Connolly *et al.*, 1998).

2.16 References

Abrams B, Duncan D & Hertz-Picciotto I (1993). A prospective study of dietary intake and acquired immune deficiency syndrome in HIV-seropositive homosexual men. **J Acquir Immune Def Syndr 6**: 949-958.

ACC/SCN (2000). The Fourth Report on the World Nutrition Situation: Nutrition throughout the lifecycle. Geneva: ACC/SCN: 1-10.

ADSA (Association for Dietetics in Southern Africa) (1996). The role of nutritional intervention in the treatment of human immunodeficiency virus infection in adults. **S Afri Foods Sci Nutr 8**: 162-164.

Allard JP, Aghdassi E, Chau J, Tam, C, Kovacs CM, Salit IE *et al.*(1998). Effects of vitamin E and C supplementation on oxidation stress and viral load in HIV-infected subjects. **AIDS 12**: 1653-1659.

Allen TM (2000). Tat-specific cytotoxic T lymphocytes selected for SIV escape variants during resolution of primary viraemia. **Nature 407**: 386-390.

Anyanwu RC & Enwonwu CO (1995). Impact of urbanization and socio-economic status on infant feeding practices in Lagos, Nigeria. **Food and Nutrition Bull 7**: 33-37.

Autran B & Katlama C (2000). Sufficient Immune Reconstitution is possible in the context of HAART: for and against. Program & Abstract of X111 Int AIDS Conf July 9-14. Durban, South Africa.

Babamento G & Kotler DP (1997). Malnutrition in HIV infection. **Gastroenterol Clin North Am 26**: 393-415.

Babameto G, Kotler DP, Burastero S (1994). Alterations in hydration in HIV-infected individuals. **Clin Res 42**: 279.

Bagasra O, Hauptman SP, Lischener HW (1992). Detection of human immunodeficiency virus type 1 provirus on mononuclear cells by in situ polymerase chain reaction. **N Engl J Med 326**: 1385-1391.

Baker J, Martin L & Piwoz E (1996). Cellular antioxidant status and human immunodeficiency virus replication. **Nutr Rev 50**: 15-18.

Barnhart HX, Caldwell MB, Thomas P (1996). Natural history of human immunodeficiency virus disease in perinatally infected children: an analysis from the Pediatric Spectrum of Disease Project. **Pediatrics 97**: 710-716.

Bartlett JG (1998). Natural history and classification of HIV infection. Chapter 2 in: Medical Management of HIV infection: 1-15. JG Bartlett (ed). Baltimore: Port City Press.

Baum MK (1992). Dietary recommendations for early stage HIV-infected persons. Amsterdam Int AIDS Conf, abstr no.3675.

Baum MK & Shor-Posner G (1998). Micronuteients status in relationship to mortality in HIV-1 disease. **Nutr Rev 51**: S135-S139.

Baum MK, Shor-Posner G & Lu Y (1995). Micronutrients and HIV-1 disease progression. **AIDS 9** (9): 1051-1056.

Beck MA (2000). Nutritionally induced oxidative stress: Effect on viral disease. **Am J Clin Nutr 71** (Suppl): 1676S-1679S.

Bell SJ, Bistrian BR, Connolly CP & Forse RA (1997). Body composition changes in patients with human immunodeficiency virus infection. **Nutrition 13**: 629-632.

Bendich A, Chandra EK (1990). Micronutrients and immune function. **New York Acad Sci 142**: 32-38.

Bentwich Z, kalinkovich A, Weisman Z (1999). Immune activation in the context of HIV infection. **Clin Exp Immunol 111**: 1-2.

Boelaert JR, Weinberg GA & Weinberg ED (1996). Altered iron metabolism in HIV infection: Mechanisms, possible consequences and proposals for management. **Infect Agents Dis 5**: 36-46.

Bogden JD, Kemp FW, Han S (2000). Status of selected nutrients and progression of human immunodeficiency virus type 1 infection. **Am J Clin Nutr 72**: 809-815.

Bouic PJD, Clark A, Brittle W, Lamprecht JH, Free-Stone M & Liebenberg RW (2001). Plant sterol/sterolin supplement use in a cohort study of South African HIV-positive patients. **S A M J 91** (10): 83-84.

Bowen D, Lane H, Fauci A (1995). Immunopathogenesis of the acquired immunodeficiency syndrome. **Ann Intern Med 103**: 704-709.

Bragbjerg IC (1993). AIDS in a Danish surgeon. **The Lancet 925**: 56-58.

Buehler JW, De Cock Km & Brunet J (1993). Surveillance definitions for AIDS. **AIDS 7** (Suppl): S73-S81.

Cahill KM (1994). The AIDS epidemic: **African Health 2**: 1-60.

Cardo M, Culver DH, Ciesielski CA (1997). A case-control study of HIV seroconversion in health care workers after percutaneous exposure. **N Engl J Med 337**: 1485-1490.

Castaldo A, Tarallo L & Palomba E (1996). Iron deficiency and malbsorption in HIV disease. **Paediatr Gastroenter Nutr 22** (4): 359-363.

Castetbon K, Kadio A, Bondurand A, Boka Yao A, Barouan C (1997). Nutritional status and dietary intakes in human immunodeficiency virus (HIV)-infected outpatients in Abidjan, Cote d' Ivoire, 1995. **European J Clin Nutr 51**: 81-86.

Cathcart RF (1991). A unique function for ascorbate. **Med Hypothesis 35**: 32.

Centers for Disease Control (1987). Revision of the CDC case surveillance definition for acquired immunodeficiency syndrome. **Monthly Morbidity Weekly Report of CDC Surveillance Summary; 36** (Suppl 1S: 3S-14

Chandra RK (1991). 1990 McCollum Award Lecture. Nutrition and immunity: lessons from the past and new insights into the future. **Am J Clin Nutr 53**: 1087-1111.

Chandra RK (1996). Nutrition, immunity and infection: from basic knowledge of dietary manipulation of immune responses to practical application of ameliorating suffering and improving survival. **Proc Natl Acad Sci USA 93**: 14304-14307.

Chandra RK (1997). Nutrition and the immune system: an introduction. **Am J Clin Nutr 66**: 460S-463S.

Cheesbrough M (1992). Medical laboratory manual tropical countries. Vol 1: 7 pp 34-56, 2^{nd} Edition, ELBS, University Press, Cambridge

Chun T, Carruth L, Finzi D (1997). Recovery of replication-competent HIV despite prolonged suppression of plasma viraemia. **Science; 278**: 1291-1295.

Cimoch P (1997). Nutritional health, Prevention and treatment of HIV-associated malnutrition: a case manager's guide. Int Assoc of Physicians in AIDS Care. www.iapac.org/clinmagt/nutrition/caseguide.html. (Accessed on 15 April 2003).

Cocchi F, DeVico A, Garzino-Demo A (1995). Identification of RANTES as the major HIV suppressive factors produced by $CD8^+$ T cells. **Science 270**: 1811-1915.

Cohen OJ & Fauci AS (2001). Host factors that affect sexual transmission of HIV. **Int J Infect Dis 2** (4): 182-185.

Connolly M, Preble EA, Sittitrai W (1998). A world perspective on HIV/AIDS and children. In: Pizzo PA and Wilfert CM, eds. Pediatric AIDS: The challenge of HIV-1 infection in infants, children and adolescents. 3^{rd} ed. Baltimore, MD: Williams & Wilkins: 112-124.

Coutsoudis A, Bobai RA and Coovadia HM (1995). The effects of vitamin A supplementation on the morbidity of children born to HIV-infected women. **Am J Public Health 85**: 1076-1081.

D'Souza MP & Harden VA (1996). Chemokines and HIV-1 second receptors. Confluence of two fields generates optimism in AIDS research. **Nature Med 2**: 1293-1300.

Dalgleish AG & Colizzi V (1992). Role of major histocompatibility complex recognition in the protection and immunopathogenesis of AIDS. **AIDS 6**: 523-525.

Dannhauser A, Van Staden AM, Van der Ryst E, Nel M, Marais N, Erasmus E *et al.* (1999). Nutritional status of HIV-1 sero-positive patients in the Free State Province of South Africa: Anthropometric and dietary profile. **European J Clin Nutr 53**: 165-173.

Darby SC, Weart DW, Giangrande PLF (1996). Importance of age at infection with HIV-1 for survival and development of AIDS in UK haemophilia population. **Lancet 347**: 1573-1579.

DeGruchy GC (1990). Clinical Haematology.In: Medical practice, 5th Edition, University Press, London: 489-493.

Denny TN, Skurnick JH, Palumbo P (1998). CD3+CD8+ cell levels as predictors of transmission in human immunodeficiency virus-infected couples: a report from the heterosexual HIV transmission study. **Int J Infect Dis 2**: 186-192.

DeVincenzi I (1994). Longitudinal study of human immunodeficiency transmission by heterosexual partners. **N Engl J Med 331**: 341-346.

Dhianraj C (2000). Institutionalising the response to HIV/AIDS in the South African University sector: A SAUVCA Analysis: 8-9.

Dorrington R, Bourne D, Bradshaw D, Laubscher, Timaeus IM (2001). The impact of HIV/AIDS on adult mortality in South Africa. Technical Report. MRC, Cape Town; 44-45.

Dushimimana A, Graham NMH, Humphrey JH (1992). Maternal vitamin A levels and HIV-related birth outcome in Rwanda. Paper presented at the 8th International AIDS Conference/111 STD World Congress, Amsterdam. The Netherlands: Abstr. POC 4221

Dworkin BM, Wormser GP, Axelrod F, Pierre N, Schwardz E & Seaton T (1990). Dietary intake in patients with acquired immunodeficiency syndrome (AIDS), patients with AIDS-related complex and serologically positive human immunodeficiency virus patients: correlations with nutritional status. **J Pop Epi Nutr 14**: 605-609.

Dworkin BM, Wormser GP, Rosenthal WS, Heier SK, Braunstein M, Weiss L *et al.* (1985). Gastrointestinal manifestations of the acquired immunodeficiency syndrome: a review of 22 cases. **Am J Gastroenterol 80**: 774-778.

Dwyer JT (1998). Dietary assessment. In: Modern nutrition in health and disease. ME Shils, JA Olson, M Shike & AC Ross (ed). 9th Edition. Philadelphia. Lea & Febiger: 942

Efem SE (1990). AIDS and surgical practice. **Nig Med Pract 19**: 83-86.

Enger C, Graham N, Peng Y (1996). Survival from early, intermediate and late stages of HIV infection. **J Am Med Assoc 275**: 1329-1334.

Enggelson E, Wang J, Pierson RN (1997). Changes in body weight and composition in HIV-infected women and men from 1984-1986. Proceedings of the Second National Conf on Women and HIV; 1: 170A.

Enwonwu CO (1992). Interface of malnutrition and human immunodeficiency virus infection in sub-Saharan Africa: a critical review. **Nutr Res 12**: 1041-1050.

Fauci A (1988). The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. **Science 239**: 617-622.

Fauci AS, Schnittman SM, Po[illegible] ([illegible]). [illegible]ational Institute of Health Conf: Immunopathogenic mechanisms in human immunodeficiency virus (HIV) infection. **Annals Int Med 114**: 678-693.

Fawzi WW & Hunter DJ (1998). Vitamins in HIV disease progression and vertical transmission. **Epidemiology 9**: 457-466.

Fawzi WW (2003). Micronutrients and HIV-1 disease progression among adults and children. Working paper for World Health Organization: 3-7.

Feinberg MB (1995). Human retrovirus infections. **Scientific Am Med 7**: 1 38.

Fleury S (1998). Limited CD4$^+$T-cell renewal in early HIV-1 infection: effect of highly antiretroviral therapy. **Nature Med 4**: 794-801.

Flodin NW (1988). Pharmacology of micronutrients, Allan Rev Liss Inc 6: 201-244.

Flores EA, Bistrian BR, Pomposelli JJ (1989). Infusion of tumor necrosis factor/cachectin promotes muscle catabolism in the rat. **J Clin Invest; 83**: 1614-1622.

Food & Dug Administration (FDA) (1995). Oral fluid specimen test for HIV-1 approved. **J Am Med Assoc 273**: 613.

Fox CH & Cottler-Fox M (1992). The pathology of HIV infection. **Immunol Today 13**: 353 356.

Fox CH (1991). Lymphoid germinal centers are reservoirs of HIV infection and account for the apparent latency of infection. **AIDS Res Hum Retroviruses 8**: 756-758.

Fox CH (1996). The pathogenesis of HIV disease. **J Nutr 126**: 2608S-26010.

Fox CH, Enner-Racz K, Racz P, Firpa A & Fauci AS (1992). Lymphoid centers are reservoirs of HIV-1 RNA. **J Infect Dis 164**: 1051-1057.

Fox CH, Hoover S, Currall VR, Bahre HI & Cottler-Fox M (1994). HIV in infected Lymph nodes. **Nature 370**: 256.

Friis H & Michaelsen KF (1998). Micronutrients and HIV infection: A review. **Eur J Clin Nutr 52**: 157-163.

Futures Group (1999). The impact of AIDS on various sectors. Paper presented at USAID's Consultative Meeting on AIDS as a development crisis: Rethinking strategies and results, Washington DC.

Gallant JE (1999). The seropositive patient-the initial encounter. HIV Clinical Management Vol 1; Medscape.

Gilks CF (1993). The clinical challenge of the HIV epidemic in the developing world. **Lancet 148**: 1037-1038.

Grimble RF (1990). Nutrition and cytokine action. **Nutr Res 3**: 193-210.

Grossman Z & Herberman RB (1997). T-cell homoestasis in HIV infection is neither failing nor blind: modified cell counts reflect an adaptive response of the host. **Nature Med 3**: 486 490.

Grunfeld C & Feingold KR (1992). Metabolic disturbances and wasting in the acquired immunodeficiency syndrome. **N Engl J Med 327**: 329-337.

Grunfeld C, Pang M, Shimizu L, Shigenaga JK (1992). Resting energy expenditure, caloric intake and short-term weight change in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. **Am J Clin Nutr 55**: 455-460.

Haase AT (1999). Population bi[illegible]ction: viral load and CD4$^+$ T-cell demographics and dynamics in lymphatic tissues. **Annual Rev Immunol 17**: 625-656.

Harakeh S, Jariwalla RJ & Pauling L (1990). Suppression of human immunodeficiency virus replication by ascorbate in chronically and acutely infected cells. **Proc Natl Acad Sci 87**: 1745-1749.

Haynes BF, Panteleo G, Fauci AS (1996). Toward an understanding of the correlates of protective immunity to HIV infection. **Science 271**: 324-328.

Hazenberg MD, Hamann D, Schuitemaker H & Miedema F (2000). T cell depletion in HIV-1 infection: how CD4+T cells go out of stock. **Nature Immunol 1**: 285-289.

Hecker LM & Kotler DP (1990). Malnutrition in patients with AIDS. **Nutr Rev 48**: 393-401.

Ho DD, Neuman AU, Perelson AS (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. **Nature 373**: 123-126.

Hu DJ, Dondero TJ, Rayfield MA (1996). The emerging genetic diversity of HIV: the importance of global surveillance for diagnostics, research and prevention. **J Am Med Assoc 275**: 210-216.

Hughes MD, Johnson VA, Hirsch MS (1997). Monitoring plasma HIV-1 RNA levels in addition to CD4+ lymphocyte count improves assessment of antiretroviral therapeutic response. **Annals Intern Med; 126**: 929-938.

John GC, Nduati RW, Mbori ND (1997). Genital shedding of human immunodeficiency virus type 1 DNA during pregnancy: Association with immunosuppression, abnormal cervical or vaginal discharge and severe vitamin A deficiency. **J Infec Dis 175**: 57-62.

Kaplan JE, Masur H, Jaffe HW (1995). Reducing the impact of opportunistic infections in patients with HIV infection. **J Am Med Assoc 274**: 347-348.

Keating J, Bjarnason I & Somasundaran S (1995). Intestinal absorptive capacity, intestinal permeability and jejunal histology in HIV and their relation to diarrhoea. **Gut 37**:623-629.

Kelly P, Musonda R, Kafwembe E (1999). Micronutrients supplementation in the AIDS diarrhoea-wasting syndrome in Zambia: A randomized controlled trial. **AIDS 13**: 495-500.

Kennedy CM, Coutsoudis A & Kuhn L (2000). Randomized controlled trial assessing the effect of vitamin A supplementation on maternal morbidity during pregnancy and postpartum among HIV-infected women. **J A I D S 24**:37-44.

Koot M, B Van't Wout A, Kootstra NA, (1996). Relation between changes in cellular load evaluation of viral phenotype and the clonal composition of virus type infection. **J Infect Dis 173**: 349-354.

Kotler DP (1992). Nutritional effects and support in the patient with AIDS. **J Nutr 122**: 723-727.

Kotler DP, Gaetz HP, Lange M, Klein EB (1984). Enteropathy associated with the acquired immunodeficiency syndrome. **Ann Intern Med; 101**: 421-428.

Kotler DP, Thea DM, Heo M (1999). Relative influences of race, sex, environment and HIV infection upon body composition in adults. **Am J Clin Nutr; 69**: 432-439.

Kotler DP, Tierney AR, Wang J & Pierson RN Jr (1989). The magnitude of body cell mass depletion determines the timing of death from wasting in AIDS. **Am J Clin Nutr 50**: 444-447.

Kotler DP, Wang J, Pierson RN (1985). Body composition studies in patients with acquired immune deficiency syndrome. **Am J Clin Nutr 42**: 1255-1265.

Kreiss J, Willerford DM, Hensel M (1997). Association between cervical inflammation and cervical shedding of human immunodeficiency virus DNA. **J Infect Dis 170**: 1597-601.

Kuritzkes DR (1999). HIV pathogenesis and viral markers. HIV Clinical Management Vol 2. Medscape, Inc: 25-30.

Lahdevirta J, Maury CPJ, Teppo AM & Repo H (1998). Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome. **Am J Med 85**: 289-291.

Leroy V, Newell ML, Dabis F (1998). International multicentre pooled analysis of late postnatal mother-to-child transmission of HIV-1 infection. **Lancet 352**: 597-600.

Levy JA (1989). Human immunodeficiency viruses and the pathogenesis of AIDS. **J Am Med Assoc 261**: 2297-3006.

Levy JA (1993). Pathogenesis of human immunodeficiency virus infection. **Microbiol Rev 57**: 183-189.

Liu H, Chao D, Nakayama E, Taguchi H, Goto M, Xin X *et al.* (2000). Insights into HIV pathogenesis. **Proc Natl Acad Sci 96**: 4581-4585.

Lucas SB, De Cock KM, Hounnou A (1994). Contribution of tuberculosis to slim disease in Africa. **Br Med J 308**: 1531-1533.

Macallan DC (1998). Dietary intake and weight loss patterns in HIV infection. In: Miller TI and Gorbach SL, eds. Nutritional aspects of HIV infection. New York: Oxford University Press.

Macallan DC (1999). Wasting in HIV infection and AIDS. **J Nutr 129**: 238S-242S.
Macallan DC, Noble C, Baldwin C (1995). Energy expenditure and wasting in human immunodeficiency virus infection. **N Engl J Med 333**: 83-88.

Macallan DC, Noble C, Baldwin C, Foskett M (1993). Prospective analysis of patterns of weight change in stage 1V human immunodeficiency virus infection. **Am J Clin Nutr 58**: 417-424.

MacDouall DS (1997). Quantitative measurement of HIV RNA: techniques and clinical applications. **J Int Assoc of Physicians in AIDS Care 2**: 9-14.

Maletnlema TN (1991). Politics and nutrition in Africa: World Health Report: 14-15.

Martensson J & Meister A (1991). Glutathione deficiency decreases tissue ascorbate levels in newborn rats: Ascorbate spares glutathione and protects Vitamin E. **Proc Natl Acad Sci 88**: 4656.

Martin D (2000). Appropriate laboratory monitoring of HIV. **S Afri Med J 90** (1): 33-35.

Maw MA (2000). AIDS epidemiology in Lesotho: Annual Report on STD and HIV/AIDS. A report: 1-22.

McCune JM (2001). The dynamics of $CD4^+$ T-cell depletion in HIV disease. **Nature 410** (19): 974-979.

McCune, IM & Kaneshima H (1995). In Human Haematopoiesis in SCID Mice (eds Roncarolo, MG, Peault B & Namikawa R): 129-156.

McDougal JS, Kennedy MS, Sligh JM (1996). The pathogenesis of HIV. **Science 231**: 382-385.

Mellors JW, Kingsley LA, Rinaldo CR (1995). Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. **Annals Intern Med 122**: 573-579.

Metcalf JA, Davey RT & Lane HC (1997). Acquired immunodeficiency syndrome: serologic and virologic tests. In: AIDS: Etiology, Diagnosis, Treatment and Prevention

Devita Jr VT, Hellman S & Rosenberg SA (eds). Philadelphia: Lippincott-Raven: 177-195.

Mocroft A, Kirk O, Barton SE (1999). Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA Study Group. **AIDS 13** (8): 943-950.

Molina JM, Scadden DR & Byrn SK (1989). Production of tumour necrosis factor and interleukin-1 beta by monocytic cells infected with human immunodeficiency virus. **J Clin Invest 84**: 733-737.

Moore JP, Trkola A & Dragic T (1997). Co-receptors for HIV-entry. **Current Opinion in Immunology 9**: 551-556.

Moore RD, Keruly LC & Chaisson RF (1998). Anaemia and survival in HIV infection. **J Acquir Immune Defic Syndr Hum Retroviral 19**: 29-33.

Murray CJ & Lopez AD (1996). The global burden disease. Cambridge: Harvard University Press.

Nduati RW, John BC, Richardson BA (1995). Human immunodeficiency virus type 1 infected cells in breast milk: Association with immunosuppression and vitamin A deficiency. **J Infect Dis 172**: 1461-1468.

Ndure KS, Sy MN & Ntiru M (1999). Best practices and lessons learned for sustainable community nutrition programming. Washington, DC: Academy for Educational Development.

Nimmagadda AP, O,Brien WA & Goetz MB (1998). The significance of vitamin A and carotenoid status in persons infected by the human immunodeficiency virus. **Clin Infect Dis 26**: 711-718.

Niyongabo T, Henzel D, Ndayishimiye JM, Melchior JC, Ndayiragije A, Ndihokubwayo JB *et al.* (1999). Nutritional status of adult inpatients in Bujumbura, Burundi (impact of HIV infection). **European J Clin Nutr 53**: 579-582.

Norse D (1991). Socio-economic impact of AIDS on food production in East Africa. Int Conf. **AIDS 7**: 71 (abstract no. TU D.57).

Nowak MA (1997). Viral dynamics of primary viraemia and antiretroviral therapy in simian immunodeficiency virus infection. **J Virol 71**: 7518-7525.

Odeleye OE & Watson RR (1991). The potential role of vitamin E in the treatment of immunologic abnormalities during acquired immune deficiency syndrome. **Prog Food Nutr Sci 15**: 1-19.

Oguntibeju OO & Banjoko O (2003). A study on the activities of liver enzymes in HIV/AIDS patients. **J Med Sciences 3** (1): 106-109.

Oguntibeju OO & Fabode F (2002). Prevalence of sexually transmitted diseases (Gonorrhoea, Trichomoniasis, Candidiasis, Syphilis) and HIV infection among young adults in Oyo, Nigeria. **Medical Technology 16** (1): 351-352.

Oguntibeju OO, Van Schalkwyk FE, Van den Heever WMJ & Veldman FJ (2003a). Importance of vitamin and mineral supplementation in HIV/AIDS patients to improve their nutritional and immunological status. **Pak J Med Sci 19** (3): 217-219.

Oguntibeju OO, Veldman D & Van Schalkwyk F (2002). Biological, social and environmental factors affecting sexual transmission of HIV infection. **Medical Technology 16** (2): 372-373.

Oguntibeju OO, Veldman D & Van Schalkwyk F (2003b). Nutritional status of people living with HIV/AIDs: The situation in Africa: Mini Review. **J Biomed & Lab Sci 15** (3): 63-67.

Ott M, Lambke B, Fischer H (1993). Early changes of body composition in human immunodeficiency virus-infected patients: tetrapolar body impedance analysis indicates significant malnutrition. **Am J Clin Nutr 57**: 15-19.

Pakker NG (1998). Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation. **Nature Med 4**: 208-214.

Pantaleo G, Graziosi C & Fauci AS (1998). The immunopathogenesis of human immunodeficiency virus infection. **N Engl J Med 328**: 327-335.

Pantaleo G, Graziosi C, Butini (1993). HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. **Nature 362**: 355-358.

Paton NIJ, Macallan DC, Jebb SA, Noble C, Baldwin C, Pazianas M *et al*. (1996). A comparison of methods for measuring changes in fat-free mass in patients with acquired immune deficiency syndrome. **Proc Nutr Soc 56**: 153A.

Paxon WA, Martin SR, Tse D (1996). Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple sexual exposures. **Nature Med 2**: 412-417.

Physicians Association for AIDS Care (1992). Nutrition and HIV/AIDS. Proceedings of the 1992 International Symposium on Nutrition and HIV/AIDS, including the Nutrition Algorithm and Nutrition Initiative of the Physicians Association for AIDS Care. Vol 1, PAAC Publishing Inc., Chicago.

Piwoz EG & Preble EA (2000). HIV/AIDS and nutrition: A review of the literature and recommendations for nutritional care and support in sub-Saharan Africa. A report: 1-22.

Pope M (1994). Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. **Cell 78**: 389-398.

Raghaven SS, Kotler DP, Loden [illegible]nces in body composition between women and men: impact of HIV and substance use. X1 Intern Conf on AIDS; 1: 125.

Rice AL (2000). Malnutrition as an underlying cause of childhood deaths associated with infectious diseases in developing countries. **Int J Publ Health 78** (10): 1207.

Roederer M (1995). CD8 naïve T cell counts decrease progressively in HIV-infected adults. **J Clin Invest 95**: 2061-2066.

Rosenberg ZF & Fauci AS (1990). Immunopathogenic mechanisms of HIV infection: cytokine induction of HIV expression. **Immunol Today 11**: 176-180.

Rowland-Jones S, Ran R, McMichael A (1997). Role of cellular immunity in Protection against HIV infection. **Adv Immunol 65**: 277-346.

Royce RA, Sena A, Cates W (1997). Sexual transmission of HIV. **N Engl J Med 336**: 1072-1078.

Saag MS, Holodniy M, Kuritzkes DR (1996). HIV viral load markers in clinical practice. **Nature Med 2**: 625-629.

Sabatier (1987). AIDS and the Third world. Norwegian Red Cross. A report: 5-75.

SAHIVCS (2001). Course in HIV/AIDS management: 5-40.

Savage KF & Burgess A (1995). Nutrition for developing countries. 2nd ed, Oxford University Press, London.

Schechter MT, Hogg RS, Aylward B, Craib KJ, Le TN, Montaner JS (1994). Higher socioeconomic status is associated with slower progression of HIV infection independent of access to health care. **J Clin Epidemiol 47**: 59-76.

Schrimshaw NS & SanGiovanni JP (1997). Synergistism of nutrition, infection and immunity : an overview. **Am J Clin Nutr 66**: 464S-477S.

Schwarz KB (1996). Oxidative stress during viral infection: A review. **Free Rad Biol Med 21**: 641-649.S.

Schwenk A, Hoffer-Belitz E, Jung B (1996). Resting energy expenditure, weight loss and altered body composition in HIV infection. **Nutrition 12**: 595-601.

Seidlin M, Vogler M, Lee YS (1993). Heterosexual transmission of HIV in a cohort of couples in New York City. **AIDS 7**: 1247-1254.

Semba RD & Tang (1999). Micronutrients and the pathogenesis of human immunodeficiency virus infection. **Br J Nutr 81**: 181-189.

Semba RD (1997). Vitamin A and human immunodeficiency virus infection. **Proc Nutr Soc 56**: 459-469.

Semba RD, Caiaffa WT, Graham NMH (1994). Vitamin A deficiency and wasting as predictors of mortality in HIV-infected drug users. **J Infect Dis 171**: 1196-1202.

Semba Rd, Lyles CM, Margolick JB (1998). Vitamin A supplementation and human immunodeficiency virus load in injection drug users. **J Infect Dis 177**: 611-616.

Serwadda D, Mugerwa RD, Sewankambo NK (1985). Slim disease: a new disease in Uganda and its association with HRLV-111 infection. **Lancet 2**: 848-857.

Smith JW & Nichols R (1991). Barrier efficiency of surgical gowns. **Arch Surg 126**: 756-759.

Smith PD, Lane HC, Gill VJ (1988). Intestinal infections in patients with the acquired immunodeficiency syndrome (AIDS). Etiology and response to therapy. **Ann Intern Med 108**:328-333.

Soderlund N, Zwi K, Kinghorn A, Gray G (1999). Preventing vertical transmission of HIV- a cos effectiveness analysis of options available in South Africa. **Br Med J 318**: 1650-1655.

Stack JA, Bell SJ, Burke PA (19[illegible]). High-energy, high-protein, oral, liquid, nutrition supplementation in patients with HIV infection: Effect on weight status in relation to incidence of secondary infection. **J Am Diet Assoc 96**: 337-341.

Stein DS, Korvick JA, Vermund SH (1992). CD4+ lymphocyte cell enumeration For prediction of clinical course of human immunodeficiency virus disease: a review. **J Infect Dis 165**: 352-363.

Suttmann U, Ockenga J, Selberg O (1995). Incidence and prognostic value of malnutrition and wasting in human immunodeficiency virus-infected outpatients. **J Acquir Immune Defic Syndr Hum Retrovirol 8**: 239-246 (abstract).

Tang Am & Smit E (1998). Selected vitamins in HIV infection: a review. **AIDS Patient Care & STDs 12**: 263-273.

Tang AM, Graham NM & Saah AM (1996). Effects of micronutrient intake on survival in human immunodeficiency virus type 1 infection. **Am J epidemiol 143**: 1244-1256.

Tang AM, Graham NM, Kirby AJ (1993). Dietary micronutrient intake and risk of progression to acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus type 1 (HIV-1)-infected homosexual men. **Am J Epidemiol 138**: 937-951.

Tang AM, Graham NMH, Semba RD, Saah AJ (1997). Association between serum vitamin A and E levels and HIV-1 disease progression. **AIDS 11**:613-620.

Task force on nutritional support in AIDS (1989). Guidelines for nutritional support in AIDS. **Nutrition 5**: 39-45.

Thea DT, Kotler DP, Engelson ES (1995). Comparison of HIV infection and other diseases upon the pattern of wasting in hospitalized African men and women. **Clin Res 43**: 254A.

Tindall B & Copper DA (199[illegible]). [illegible] infection: host responses and intervention strategies. **AIDS 5**: 1-14.

Trujillo EB, Borlase BC & Bell S (1992). Assessment of nutritional status, nutrient intake and nutrition support in AIDS patients. **J Am Drug & Adm 92** (4): 477-478.

Turk DC, Porter IA, Duuerden BI & Reid TMS (1993). A Short Textbook of Medical Microbiology, Parts 111 and V. Hodder and Stoughton (eds) ELBS, 5th ed: 121-128.

Ullum H, Lepri A, Victor J (1998). Production of beta chemokines in Human immunodeficiency virus (HIV) infection: evidence that high levels of macrophage in inflammatory protein-1 beta are associated with a decreased risk of HIV disease progression. **J Infec Dis 177**: 331-336.

UNAIDS (1999). Differences in HIV spread in four sub-Saharan African cities.A report 14 September: 1-6.

UNAIDS (2000). Global HIV/AIDS epidemic. UNAIDS Fact sheet. 1-26.

UNAIDS (2001). Global HIV/AIDS epidemic. UNAIDS Fact sheet. 1-29.

UNAIDS (2002). Global HIV/AIDS epidemic. UNAIDS Fact sheet. 1-26.

USPH/IDSA Prevention of Opportunistic Infections Working Group (1996). USPH/IDSA guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus: a summary. **Ann Intern Med 124**: 346-361.

Van Staden AM, Barnard HC, Nel M, Attwood EM, Dannhauser A, Van der Ryst E *et al.* (1998). Nnutritional status of HIV-1 sero-positive patients in the Free State Province of South Africa-laboratory parameters. **Cent African J Med 44** (10): 246-250.

Vogt MW, Witt DJ, Craven DE (1987). Isolation patterns of the human immunodeficiency virus from cervical secretions during the menstrual cycle of women at risk for the acquired immunodeficiency syndrome. **Ann Intern Med 106**: 380-382.

Vonroenn JH, Murphy RL, Weber KM (1998). Magesterol acetate for treatment of cachexia associated with human immunodeficiency virus infection. **Ann Intern Med 109**: 840-841.

Watson RR (1994). Nutrition and AIDS. CRC Press, Ann Arbor, London: 118-130.

Wawer MJ, Sewankambo NK, Serwadda D (1999). Control of sexually transmitted diseases for AIDS prevention in Uganda: a randomised community trial. Rakai Project Study Group. **Lancet 353** (9152): 525-535.

Wei X, Ghosh SK, Taylor ME (1993). Viral dynamics in human immunodeficiency virus type 1 infection. **Nature 373**: 117-122.

Weir DM (1993). Immunology: An Outline for Students of Medicine and Biology. Church Livingstone. ELBS, 5th ed. 24-42.

Weiss R (1996). HIV receptors and the pathogenesis of AIDS. **Science; 272**: 1885-1886.

Wheeler DA, Gibert C, Launer CA (1998). Weight loss as a predictor of survival and disease progression in HIV infection. **J Acquir Defic Syndr 18**: 80-85.

Wilson CB (1990). The cellular immune system and its role in host defense. New York, NY: Churchill Livingstone Inc: 101-138.

Wong JK, Hezareh M, Gunthard HF (1997). Recovery of replication-competent HIV despite prolonged suppression of plasma viraemia. **Science 278**: 1291-1295.

World Health organization (19[illegible]). World Health Organization (WHO) global programmes on AIDS. Proposed WHO staging system for HIV infection and disease. **AIDS 7**: 868-869.

Woznicki D & D'Alessandro (1997). Nutrition against AIDS. **Am Council Sci & Health 9**: (2): 1-3.

Young J (1997). HIV and medical nutrition therapy. **J Am Diet Assoc 97** (10) (Suppl 2): S161-167.

Zangerle R, Reibnegger G, Wachter H, Fuchs (1993). Weight loss in HIV-1 infection is associated with immune activation. **AIDS 7**: 175-181.

Zhang ZQ (1998). Kinetics of $CD4^+$ T-cell population of lymphoid tissues after treatment of HIV-1 infection. **Proc Natl Acad Sci USA 95**: 1154-1159

3 METHODOLOGY

3.1 Introduction

In chapter 3, methods such as a socio-demographic questionnaire, a food frequency questionnaire, an anthropometric information sheet and a medical examination information sheet, employed in the collation of relevant information from patients, are described. The chapter also includes various procedures and tests that were applied in the determination of specific immunological and haematological variables. Operational definitions are provided for the purpose of clarity.

3.2 Operational Definitions

For the purpose of clarity, certain operational terms used (or what they constitute) in this study are explained below:

3.2.1 Socio-demographic Status (Appendix A)

For this study, socio-demographic status constitutes the following factors:

- Age (in years), gender and number of years resident in Bloemfontein
- Marital status and number of children (alive or dead)
- Smoking habits
- Level of education
- Employment status
- Financial status and number of people contributing to income per month
- Type of house and available cooking utensils
- Number of people living in the house and amount spent on food monthly/weekly

3.2.2 Anthropometry

Anthropometry involves obtaining physical measurements of an individual, and relating these measurements to standards that also reflect his/her health and nutritional status, such as malnutrition (Lee & Nieman, 1996; Gibson, 1998). The most commonly used anthropometric measurements include body weight, height, hip circumference, waist circumference, skinfold thickness and other girth measurements.

- A waist-to-hip circumference ratio of <0.95 and ≥ 0.95, indicative of male fat distribution (Hammond, 2004) and a waist-to-hip circumference ratio of < 0.8 (female), indicative of a gynoid fat distribution.
- Waist circumference was categorised as follows: < 0.88: Normal waist circumference; ≥0.88: High waist circumference (female) (Smolin & Grosvenor, 2000).

- Weight (W) and Height (H) measurements were obtained to determine the body mass index (BMI) as W/H^2, with weight measured in kilograms and height measured in square metres (Laquatra, 2004).

BMI: <18.5 (underweight)
BMI: 18.5-24.9 (average weight)
BMI: ≥25 (overweight) (Jackson & Pollock, 1985)

- Fat percentage was categorised as follows (Laquatra, 2004)
  Low: <20
  Normal: 20≤25
  High >25

- Skinfold (SKF) Thickness

The skinfold thickness refers to the thickness of a double fold of skin and compressed subcutaneous adipose tissue (Gibson, 1998). This measurement relies on total body fat estimates on the assumption that 50% of body fat is subcutaneous (Hammond, 2004).

3.2.3 Dietary Intake

For dietary intake, reference is made to the habitual types, quantities and frequency of food and drink consumed on a daily, weekly or monthly basis over a period of six months prior to the study. A validated food frequency questionnaire (adapted from the Transition and Health During Urbanisation of South Africans (THUSA) study (Potchefstroom University), was used (Appendix C) to determine the habitual intake of the energy, macro- and micronutrients mentioned below:

- Energy and macronutrients (carbohydrates, fats and proteins)
- Vitamins (vitamins A, C, E, D, K, B_6, B_{12}, folic acid, niacin, thiamine and riboflavin)
- Minerals and trace elements (magnesium, chromium, phosphorus, iodine, iron, selenium, zinc)

The intake of each nutrient is considered to be either less than 67% or greater than 67% of the recommended allowance (RDA) or adequate intake (AI)

3.2.4 Immunological and Haematological Parameters

- Total T-cell count (940-2380 cells/mm^3)
- $CD4^+$T-cell count (510-1310 cells/$mm^{3)}$
- CD8+T-cell count (510-1310 cells/mm^3)
- CD4/CD8 ratio (0.72-3.14)
- Viral load (>10 000/ml)
- Red cell count (3.7-5.3 $x10^9$/l for female and 5.5-9$x10^9$/l for male)
- Haemoglobin (12-16 g/dl for female and 13-18 g/dl for male)
- Haematocrit (0.35-0.45l/l for female and 0.40-0.50l/l for male)
- Mean cell haemoglobin concentration (MCHC) (32-37 g/dl for female and 32-36 d/dl for male)
- Mean cell concentration (MCH) (29.5-32 pg)
- Mean cell volume (MCV) 86-90 fl)
- Red distribution width (RDW) (10-15)
- White cell count (WCC) (7.5-11 $x10^9$/l)
- Neutrophil (2-75 $x10^9$/l)
- Lymphocyte (1-4 $x10^9$/l)
- Monocyte (0-0.95 $x10^9$/l)

- Eosinophil (0-0.4 x10^9/l)
- Basophil (0.-0.1 x10^9/l)
- Platelets (150-400 x10^9/l)

3.3 Type of Study

This was an open-labelled, clinical trial study that involved HIV-positive/AIDS individuals (volunteers). A total of 50 HIV-positive/AIDS patients were recruited for the study after signing the consent form.

3.4 Study Population

The target subjects for this study were volunteers living with HIV/AIDS. In the study population, females and males were included within the age group of 18 to 65 years. None of the subjects were on anti-retroviral therapy or any treatment for chronic diseases. No control group was used for this study due to the following reason: The $CD4^{+}$T/$CD8^{+}$T-cell counts of the inclusion criteria of the patients were very low and an untreated control group would have too much drop-out owing to death and terminal illness.

The data of each patient's $CD4^{+}$T/$CD8^{+}$T-cell counts and viral load count determined during the screening visit were treated as an internal control. In different studies on the nutritional status and dietary intake of HIV-positive/AIDS patients, Coodley *et al.* (1993), Castetbon *et al.* (1997), and Van Staden *et al.* (1998), did not include control groups.

3.4.1 Sample Size

Fifty patients selected after the screening visit, who met the inclusion criteria, who did not meet any of the exclusion criteria, and who gave written informed consent, were recruited into the study.

3.4.2 Justification for In and Exclusion Criteria

The criteria were set to ensure a homogenous subject population for this study.

3.4.3 Inclusion and Exclusion Criteria

Patients had to:

3.4.3.1 Inclusion Criteria

- Be male and female subjects from 18 to 65 years of age and HIV/AIDS positive.
- Have $CD4^{+}$T-cell counts of 100 – 350 cells/mm^3
- Display symptoms within the range of clinical acceptability in medical history and physical examination, and laboratory results acknowledged by the clinical investigator.
- Be willing to undergo a pre-study physical examination and pre- and post study laboratory investigations.
- Be able to comprehend and be willing to sign the statement of informed consent.

3.4.3.2 Exclusion Criteria

Patients were excluded on grounds of:

- Evidence of psychiatric disorder, antagonistic personality, poor motivation to participate in this study or limited ability to comply with protocol requirements.
- A 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 supplement within eight weeks before the first administration of the study medication.
- A history of hypersensitivity to the study supplement or any related drugs.
- Loss of blood equal to or exceeding 500 ml during the 8 weeks before the administration of the study medication.
- Heavy smoking (i.e. more than 20 cigarettes per day).
- Being pregnant.
- Being diabetic HIV-positive individuals.
- Being on antiretroviral therapy or any treatment for chronic diseases

3.4.4 Withdrawal Criteria

Subjects were informed of their right to withdraw from the study at any time, irrespective of the reason. The following incidents may lead to withdrawal:

- An adverse event as a result of taking the study supplement.
- Protocol violation by the patients; at the discretion of the clinical investigator.

3.4.5 Subject Identification

- Each enrolled subject received a number (01-50) and retained this number throughout the study.
- Each enrolled subject retained his initials obtained from a copy of his identification book.
- Each enrolled subject was identified by date of birth.

3.4.6 Ethical Approval

Written approval for the final version of the protocol was obtained from the Ethics Committee (Ethics number ETOVS 32/03) of the Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa before the first administration of the study supplement.

Before commencement of the screening procedures, the subjects were informed verbally and in writing by the clinical investigator about the nature and purpose of, and possible risks involved in, the screening procedures as well as the, procedures, restrictions, obligations, remuneration, insurance coverage and possible adverse supplement reaction relevant to the study. Both the informed consent discussion and the written patients' information were included to provide adequate information for the subjects and the subjects in turn voluntarily accepted the terms of the study, and agreed to co-operate in its conduct by signing the informed consent form (Appendix E).

The statement of informed consent was personally signed and dated by the subject and by the clinical investigator who conducted the informed consent discussion, after sufficient time for deliberation had been provided. The subject information sheets and informed consent forms were available in English, Afrikaans and Sotho. Each volunteer was provided with the subject information sheet and informed consent form in the language of his/her choice and retained copies of the subject information sheet and signed statement of informed consent. The subjects were also to be informed timeously should new information become available that might influence their willingness to continue participating in the study.

3.4 Study Design

3.5.1 The Screening visit

The study consisted of a screening visit and monthly visits from April to September 2003. The monthly visit started 7 days after the screening visit. The duration of this study was considered suitable and was similar to the duration reported by Allard *et al.* (1998) in a previous work. Validated questionnaires (food frequency questionnaire, socio-demographic questionnaire) were used in obtaining information from the patients. The full blood count (Dacie & Lewis, 1985), CD4$^+$T-cell count, CD8$^+$T-cell count (Romeu *et al.*, 1992; Mulder *et al.*, 1996), anthropometric measurements (Lee & Nieman, 1996) and viral loads (Twigg *et al.*, 1999) were determined using standard procedures. The methods applied in the determination of the above-mentioned laboratory parameters are described under laboratory investigations. The framework of the study is presented in figure 3.1.

3.5.2 Follow-up Visits (Monthly visits)

The patients were seen at the Medi Inn clinic on a monthly basis. During each visit, physical and medical examinations were carried out on the patients by the clinical investigator. Blood samples were drawn from the patients and full blood count, CD4$^+$T-cell counts, CD8$^+$T-cell counts were performed. The viral load and anthropometric measurements were repeated at the end of the study using standard procedures. Compliance with the regime was ensured by counting the supplement units on a daily basis and by constantly reminding the patients of the need to follow the protocol.

All data from the screening, clinical evaluation and the post study physical examination and laboratory investigations were documented. Each subject was carefully monitored for adverse events, including abnormal laboratory investigations. In addition, information on possible adverse events following [illegible] was obtained from the subjects by regular questioning of each subject by the clinical staff, although no leading questions were asked. There was no complaint of adverse events having occurred during the period of this study. The framework of the study is presented in figure 3.1.

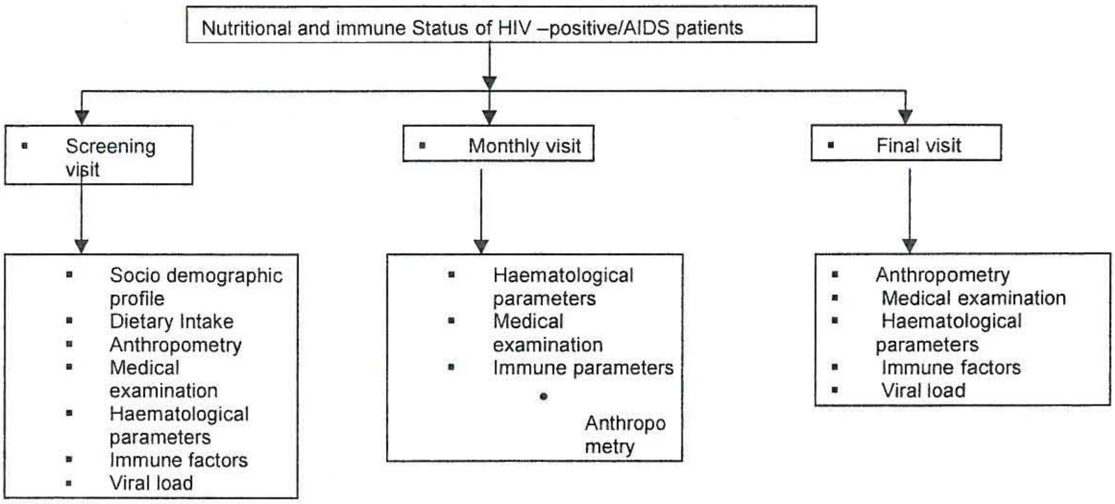

Figure 3.1: Framework of the study design to determine the nutritional and immune status of HIV-positive/AIDS patients.

3.6 Measuring Techniques and Procedures

All the apparatus, techniques and procedures applied in this study were standardised. Methods were also standardised to ensure validity and reliability of results.

3.6.1 Socio-demographic Status

3.6.1.1 Socio-demographic Measurement

Socio-demographic details such as age, gender and residential areas were obtained from each patient by means of a questionnaire. Information concerning financial and employment status, level of education, marital status, monthly income, type of house, amount spent weekly or monthly on food, available cooking facilities, smoking habit, number of children (alive or dead), and number of persons living in the house was also obtained and recorded (Appendix A). Due to small sample size, the statistical power of this data is not sufficient and will not be discussed further in this study.

3.6.2 Anthropometry

In this study the restricted profile was adopted (Schmidt & Carter, 1990). Sites measured for the restricted profile are skinfolds and include triceps, subscapular, biceps, iliac crest, abdomen, front thigh, medial calf, med-axilla, gluteal (hips) and waist. Anthropometric indices were determined by measurement.

3.6.2.1 Anthropometry Apparatus

The following anthropometric equipments was used in the anthropometric measurement: anthropometric tape: a flexible steel tape calibrated in centimetres with millimetre gradations; a scale, and skinfold callipers.

(i) Metal Tape

This was fitted to the wall and the height of each patient was taken (without shoes) to the nearest 0.1 cm.

(ii) Scale

The 770 Seca digital electronic scale (Bizerba 75860) was used in determining the body mass of the respondent to the nearest 0.1 kg.

(iii) Skinfold Callipers

Slim guide callipers were used as recommended (Schmidt & Carter, 1990).

3.6.2.2 Anthropometric Measurements and Data Collection

Subjects were informed as to what measurements were to be taken and were asked to complete a consent form as part of the preliminaries of the test protocol. Throughout the marking and measurement session, each subject stood relaxed, with arms comfortably by the side and feet held together. However, some measurements required the subjects to place their feet apart. The subjects presented themselves in minimal clothing to allow for measurements to be done quickly and efficiently. Previous study has shown that skin-fold thickness is reliable (Paton *et al.*, 1996). All anthropometric measurements were done according to standard procedures (Lee & Nieman, 1996).

Two persons were involved with the measurement: the measurer and the recorder. This was done to ensure 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 measurement was done twice at each site on each subject and the average value taken. The various measurements that were taken and the procedures involved are described below.

(i) Height, Weight & Body Mass Index

Height and weight can be measured using different approaches and are useful in determining nutritional status in adults (Pressman & Adams, 1990; Gibson, 1998).

Stature or standing height can be measured in various ways (Lee & Nieman, 1996). The simplest method that can be applied, is to fasten a measuring stick or non-stretchable tape measure to a flat, vertical surface, for instance, a wall without a skirting, with the subject not standing on a carpet (Lee & Nieman, 1996). Body weight, which provides a crude evaluation of overall fat and muscle stores forms one of the most important measurements in nutritional assessment.

It is indicated in literature that usual body weight compares the present weight of the individual to the usual body weight, thus allowing changes in body weight to be assessed (Hammond, 2004). Actual body weight refers to weight measurement obtained at the time of examination.

(a) Procedure

Body weight in this study was measured by means of an electronic load cell scale to the nearest 0.1 kg with patients dressed in light clothing, and height (without shoes) to the nearest 0.1 cm, using a metal tape fitted to the wall. The values of the body weight and height in metres were applied to calculate the BMI as BMI =W/H^2 (Pressman & Adams, 1990; Laquatra, 2000).

(ii) Body Composition

The interest in body composition has mounted over the past few years because of its relevance to health and diseases (Lee & Nieman, 1996). Body composition is defined as the ratio of fat to fat-free mass (Lee & Nieman, 1996; Gibson, 1998). The fat-free mass refers to tissue devoid of all extractable fat and is often expressed as a percentage of body fat. These two components can be indirectly assessed by anthropometric techniques, and the variations in their amount and proportion can be used as indices of nutritional status (Gibson, 1998).

Body composition can be determined using skinfold thickness, bioelectric impedance and other available methods. For the present study, skinfold thickness was used to determine the body composition of patients.

(iii) Skinfold (SKF) Thickness to determine Body Fat Percentage

Skinfold thickness is a widely used indirect method of assessing the amount of body fat. Although more accurate methods for assessing percentage body fat are available, skinfold measurement is easy and quickly obtained. It provides estimates of body composition that correlate well with those derived from hydrostatic weighing (Lee & Nieman, 1996).

The thickness of subcutaneous adipose tissue varies widely among different skinfold sites within individuals, and for the same skinfold site between individuals, overall subcutaneous adipose tissue is best assessed by taking measurements at different sites. A minimum of three different sites is recommended (Lee & Nieman, 1996). Skinfold sites identified as most reflective of body fatness are over the triceps and the biceps, below the scapula, above the iliac crest and on the upper thigh (Hammond, 2004).

(a) Procedure

The body fat percentage was determined from the 7 skinfolds: chest + abdomen + thigh + triceps + subscapular + iliac crest + midaxillary, according to the method of Jackson & Pollock (1985), by using the following formula:

Male (18-61 years): BD (body density) = 1.112 – 0.00043499 (Σ7SKF) + 0.00000055 (Σ7SKF)^2 – 0.00028826 (age). Fat percentage = [(4.85/BD) – 4.39] x 100.

Female (18-55 years): BD (body density) = 1.0970 – 0.00046971 (Σ7SKF) + 0.00000056 (Σ7SKF)2 – 0.00012828 (age). Fat percentage= [(4.37/BD) – 3.93] x 100.

(iv) Waist-to-Hip Circumference

The waist-to-hip ratio (WHR) is an easy method of assessing body fat distribution. It provides an index of regional body fat distribution and forms an important guide in assessing health risk (Lee & Nieman, 1996). The waist-to-hip ratio differentiates between android and gynoid obesity, and is the most frequently used method to measure adiposity. According to Hammond (2004), a WHR $\geq$ 1.0 in men or $\geq$ 0.8 in women is indicative of android obesity and seen as increasing the risk for obesity-related diseases. Plastic or steel measuring tapes may be used to determine the measurements. The waist circumference is measured at the most narrow area below the rib cage and above the umbilicus as viewed from the front. The subject to be measured is dressed in light clothing, stands erect, with abdominal muscles relaxed, arms by the side and feet together. The measurer then places the tape in a horizontal plane and measures the area of least circumference. The measurement is taken at the end of a normal expiration.

The hip circumference is the point of greatest circumference around the hips at the point of greatest circumference, and the measurement was taken with the tape in close contact with the skin, but without indenting the soft tissues. The measurement was recorded to the nearest 0.1 cm. The WHR was calculated by dividing the waist circumference by the hip circumference (Lee & Nieman, 1996).

(a) **Procedure**

A metal tape was used to determine the circumference of the hip and the waist at the level of the umbilicus. The mean of the measurements was recorded for each parameter and the results for waist and hip circumference were used to calculate the waist-to-hip ratio (WHR) as WHR =Waist girth/gluteal (hip) girth (Hammond, 2004).

(v) **Lean Body Mass**

(a) **Procedure**

Lean body mass (fat free mass) (FFM) was determined using the method as described by Niyongabo *et al* (1997) using the formula: LBM= Body weight-percentage body fat. The various measurements that were taken are described below.

3.6.2.3 Anatomical Landmarks

The landmarks are identifiable skeletal points that generally lie close to the body's surface and are the markers which identify the exact location of the measurement site or from which a soft tissue is located.

The landmark was identified with the thumb or index finger. The site was released to remove any distortion of the skin, then relocated and marked directly over the landmark. The mark was then checked to ensure that there had been no displacement of skin relative to the underlying bone. All landmarks were identified and marked before measurements were made (Schmidt & Carter, 1990).

(i) **Acromiale**

The acromiale is the point at the superior and lateral border of the acromion process, midway between the anterior and posterior borders of the deltoid muscle when viewed from the side of the patient.

With the measurer standing behind and on the right hand side of the subject, the subject was palpated along the spine of the scapula to the corner of the acromion. The straight over the triceps muscle when viewed from the side. For measurement, the arm was relaxed with the shoulder joint slightly externally rotated and elbow extended from the side of the body.

(vi) Subscapular

The subject was standing erect with arms by the side. The thumb was applied in palpating the inferior angle of the scapula to determine the undermost tip, and the marking made. The skinfold was raised with the left thumb and index finger at the marked site 2 cm along a line running laterally and obliquely downwards from the subscapulare landmark at an angle of 45^0 as determined by the natural fold lines of the skin.

(vii) Biceps

This skinfold was raised with the left thumb and index finger on the marked mid-acromiale-radiale line so that the fold runs vertically, that is parallel to the axis of the upper arm. The measurement was made with the subject standing with the arm relaxed, the shoulder joint slightly externally rotated and elbow extended (Schmidt & Carter, 1990).

(viii) Iliac Crest

This skinfold is raised immediately superior to the iliocristale on the ilio-axilla line. The subject was told to place the left arm across the chest to the right hand on the iliocristale landmark and exert pressure inwards so that the fingers roll over the iliac crest. The left thumb was substituted for these fingers and the index finger was relocated a sufficient distance superior to the thumb so that this grasp became the skinfold to be measured.

(ix) Supraspinale

This skinfold was originally named suprailiac by Heath & Carter (1967), but it is now known as the supraspinale (Carter & Heath, 1990). This fold is raised at the point where the line from the iliospinale mark to the anterior axillary border intersects with the horizontal line of the superior border of the ilium at the level of the iliocristale and this is about 5 cm above the iliospinale, depending on the size of the adult subject.

(x) Abdomen

This is a vertical fold raised 5 cm from the right hand side of the omphalion (midpoint of the navel). For maximal measurement of this site, it was ensured that the initial grasp was firm.

(xi) Front Thigh

The measurer stood facing the right side of the subject on the lateral side of the thigh. The subject while seated, was asked to bend his/her knee at right angles by placing the right foot. The site was marked parallel to the long axis of the femur at the mid-point of the distance between the inguinal fold and the superior border of the patella. The skinfold measurement was taken while the knee is bent

(xii) Medial Calf

With the subject in a seated position and the calf relaxed, the vertical fold was raised on the medial aspect of the calf at a level where it has maximal circumference and the point marked; the measurement was taken from the most medial point.

(xiii) Chest

This girth was taken at the level of the mesosternale. The measurer stood to the right of the subject who slightly abducts the arms allowing the tape to be passed around the chest in a near horizontal plane. The subject was told to breathe normally and the measurement was taken at the end of a normal expiration (end tidal) with the arms relaxed at the sides.

(xiv) Waist

For this measurement, the measurer stood in front of the subject to correctly locate the narrowing of the waist, and the measurement was taken at the level of the narrowest point between the lower costal (rib) border and the iliac crest.

(xv) Gluteal (Hip)

This was taken at the level of the greatest posterior protuberance of the buttocks which usually corresponds anteriorly to about the level of the symphysis pubis. The measurer stood at the side of the subject to ensure the tape is held in a horizontal plane when measuring this site. The subject stood with the feet together with gluteal muscle relaxed.

(xvi) Forearm

The measurement was taken at the maximum girth of the forearm with the subject holding the palm up while relaxing the muscles of the arm (distal to the elbow) (Schmidt & Carter, 1990).

(xvii) Thigh

The girth of the thigh was taken 1 cm below the level of the gluteal fold, perpendicular to the axis of the thigh. The subject stood erect with feet slightly apart and mass equally distributed on both feet.

(xviii) Calf

This is the maximum girth of the calf. The subject stood facing away from the measurer in an elevated position as this position allows the measurer to align the eyes with the tape. The measurement was taken from the lateral aspect of the leg by placing the tape around the calf in the prescribed manner. The maximal girth was found by using the middle fingers to manipulate the position of the tape in a series of up or down measurements. The level was marked on the medial aspect of the calf (Schmidt & Carter, 1990).

3.6.2.4 Precautions

The following steps were taken to reduce errors and obtain accuracy in anthropometric measurement:

- It was ensured that the calipers were accurately measuring the distance between the centre of their contact faces by using the short blades of the caliper. It was also ensured that the tension of the jaws of the calipers remained constant throughout the range of measurement.

- The skinfold sites were carefully located using the correct anatomical landmarks and each measurement was repeated twice and the average taken.
- The skinfold was raised at the marked line and grasped in a such way that a double fold of skin plus the underlying subcutaneous adipose tissue was held between the thumb and index finger.
- The nearest edge of the contact faces of the calipers was applied 1 cm lateral to the thumb and finger because if the calipers are placed too deeply or too shallow incorrect values would be recorded. The calipers were held 90^0 to the surface of the skinfold site during the entire period of the study and the measurement recorded two seconds after the full pressure of the calipers.
- Skinfold thickness was measured in succession to avoid experimental bias (Schmidt & Carter, 1990).

3.6.3 Dietary Intake

At baseline, a validated food frequency questionnaire (adapted from the Transition and Health During Urbanisation of South Africans [THUSA] study [Potchefstroom University] was used (Appendix C) to determine the habitual types and quantities of food and drink consumed by respondents over the six months prior to data collection, and to determine the habitual intakes of total energy, macronutrients and micronutrients. The food frequency questionnaire (FFQ) was administered by the researcher and four other team members, after attending a training session delivered by an invited dietician. Both traditional and western foods were included in the food frequency questionnaire. A food frequency questionnaire was chosen to determine the dietary intake because it is believed to be a suitable method to use for describing the intake of groups rather than for individuals (Dwyer, 1998), and is commonly used in epidemiological studies on the relation between diet and disease (Willett, 1990; Paton *et al.*, 1996). It also provides an overall picture of food intake (Dwyer, 1998; Hammond, 2000), which may be more representative of the usual food intake than a few days of diet records, and it is relatively inexpensive. The reproducibility of the FFQ was not measured due to the fact that the instrument was only used to asses baseline intake of the sample population. No follow-up data was collected.

Food models were used so as to assist with the accuracy of the size of food portions described by the respondents. Each respondent was asked to demonstrate the quantity of a given food that he/she consumed on a daily, weekly or monthly basis via an interview. The researcher and other four members of thye research team completed the food frequency questionnaire. Local interpreters were used for patients who could not speak English.

The portion sizes were estimated using hold measures and converted to grams using the conversion figures in the NRIND Food Quantities Manual (Langenhoven *et al.*, 1998). The quantities of food consumed on a daily basis were entered accordingly. The quantities of foodstuffs not selected by the respondents per day, were 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 applying the MRC food composition tables. The energy intake was compared to the estimated energy requirement. Macro-and micronutrient intakes were compared with the recommended daily allowances (RDA) or Adequate (AI) (USA Food and Nutrition, 1989 and 2001). A value of <67% of the RDA/AI was considered to be inadequate (Sappey *et al.*, 1994).

3.6.4 Study Supplement

3.6.4.1 Supplement Ingredients

Extract of Hypoxis	500 mg
Grape Fruit Seed Extract	4 mg
Sitosterol & Sitosterolin	28 mg
Beta Carotene	1 mg
Vitamin E	12.5 mg
Vitamin B_6	7.5 mg
Vitamin B_1	3.75 mg
Vitamin B_2	10 mg

Vitamin B_{12}	3 µg
Nicotinamide	5 mg
Vitamin C	50 mg
Olive Green Leaf Extract	35 mg
Folic Acid	325 µg
Natural Anti-Oxidant (Biocydin)	52 mg

3.6.4.1.1 Motivation for Inclusion of each Ingredient in the Supplement

According to Professor HC Barnard (Bermins Manual, 2004), the following motivations are provided for inclusion of each ingredient in the supplement:

(i) **Extract of Hypoxis**: The anti-HIV and anti-inflammatory properties of rooperol in the extract of the African potato in Africa's Solution is of great benefit. It brings about a feeling of well-being and slows down the progression of the disease in the HIV-positive person.

(ii) **Grapefruit Seed extract (GSE)**: It has been proven that grapefruit seed extract kills over 800 assorted bacteria and virus strains. When compared to 30 effective antibiotics and 18 proven fungicides, GSE has proven to perform as well as any and all agents tested, without exhibiting the harmful side effects usually associated with antibiotic use. It is a powerful but gentle, effective, non-toxic agent of broad-spectrum application.

(iii) **Plant sterols (Sitosterol and Sitosterolin)**: Reports from scientific papers indicate that plant sterols have a beneficial effect on the immune system (they enhance the production of lymphocytes (T-cells), interleukin II and interferon n-alpha. This immune system modulating effects of the plant sterols, mainly beta-sitosterol and beta-sitosterolin is seen by some scientists as an important function of yet another essential micro-nutrients which fits the definition of a vitamin.

(iv) **Beta-carotene**: Is a known lipid soluble anti-oxidant. It can be concluded that beta-carotene together with vitamin E protect the tissues of the body including the white blood cells against free radical damage during the oxygen burst of the inflammation process after an infection.

(v) **Vitamin E**: Is the most important biological anti-oxidant. It prevents lipid peroxidation, free radical scavenger and regulation of metalloenzymes such as super-oxide dismutase, catalase, glutathione peroxidase.

(vi) **Vitamin B_6**: Is a co-enzyme of aminotransferases, deaminases and decarbohydrates involved in all amino-acid reactions, biosynthesis of neuro transmitters.

(vii) **Vitamin B_1**: Is a coenzyme for alpha-keto acid decarboxylases and transketolases in involved in energy production, anti-oxidant generation and nucleic acids synthesis.

(viii) **Vitamin B2**: Is a coenzyme of L-amino acid oxidase, D-amino acid oxidase, dehydrogenase, vitamin K and cytochrome oxidase. It is involved in energy production.

(ix) **Vitamin B_{12}**: Many studies of people with HIV in the USA and Africa reported low serum B12 levels even among asymptomatic persons. Low serum B12 levels are associated with neurological abnormalities, for example neuropathy, impaired cognition and reduced $CD4^{+}$T-cells, hence its inclusion in the supplement.

(x) **Nicotinamide**: Is a coenzyme involved in glucose-6-Phosphate dehydrogenase, glutamate hydrogenase and Krebs cycle. As electron transport carrier.

(xi) **Vitamin C**: Vitamin C has been found to stimulate the production of interferons, the proteins that protect cells against viral attacks. It can also stimulate the positive chemotactic and proliferative responses o neutrophils. It has been shown that vitamin C can stimulate the synthesis of the humoral thymus factor and antibodies of the IgG and IgM classes. A striking property of vitamin C is its ability to inactivate viruses and inhibit viral growth in their host cells.

(xii) **Olive Green Leaf Extract**: The active substance in Olive Green Leaf Extract is oleuropein. It is believed to be an excellent fat-soluble anti-oxidant. Research articles indicate oleuropein protect LDL from oxidation. In vitro studies have shown that oleuropein offer good membrane protection properties as an anti-oxidant. Oleuropein like grapefruit seed extract is a natural wide spectrum anti-bacterial, anti-viral and anti-fungal agent. It is the only known lipid anti-oxidant which influences the activity of membrane-bound enzymes which function in the cytoplasm of the cell.

(xiii) **Folic Acid**: Is required for the enzymes that produce DNA for replicating and growing cells, including those of the gastrointestinal (GI) tract, blood and growing foetus. Deficiency results in impaired cell division and protein synthesis, causing megaloblastic anaemia. If the GI tract is damaged as is common in

HIV-related diarrhea, folic acid reabsorption may be impaired, setting off a cycle in which deficiency results in further GI tract deterioration and malabsorption of other nutrients.

(xiv) **Biocydin**: Proanthocyanidins, previously call condensed tannins are chemically very similar. They only differ in molecular size (ranging from individual units to complex molecules of many link units), shape and attachments of the polyphenol rings. These plant flavonoids are found in many woody plants. The best study and well known oligomeric proanthocyanidins are those extracted from the white pine of southern Europe (Pinus maritime, P. pinaster) and grape seeds. Grape seeds extracts have 7 to 15 percent more proanthocyanidins and has been reported to be more potent and therefore, more economical. It is clear that the proanthocyanidin content and anti-oxidant activity differ greatly from extracts of the same plant in different parts of the world in some cases. The proanthocyanidin content and anti-oxidant activity from the South African pine bark extract is very low. The highest anti-oxidant potency is found from pine bark extracts from southern Europe and Canada. In the past 10 years, the proanthocyanidin content and anti-oxidant activity was under investigation and a high proanthocyanidin and anti-oxidant activity was found in an extract from the bark of an indigenous South African free growing in the semi desert region of the country. This extract is commercialised and called biocydin. It is reported that the anti-oxidant activity is up to three times higher than those of the pine bark extract.

3.6.4.2 Supplementation

Following the determination of baseline parameters at the screening visit, the patients were given 7.5 ml of study supplement between 07:00 and 09:00 and 7.5 ml of study supplement between 16:00 and 19:00 per day. Staff members of the South African Red Cross Community Home Based Care Program, in the community, handled the dosing and monitoring of the supplement intake and compliance on daily basis for three months.

It was ensured that records of the receipt and administration of the study supplement were kept and that the supplement was not used for purposes other than as directed by the protocol. On the completion of the study, the remainder of the study supplement (with the exception of the retention sample) was returned to the sponsor, together with a letter of acknowledgement of receipt. This document was signed by the sponsor and thus formed part of the supplement file documentation and it was archived as such. The retention sample, supplied by the sponsor was stored for a period of time in accordance with the sponsor's requirements. Patients carried on with their normal diet.

3.6.5 Laboratory Investigations

3.6.5.1 Blood Sampling

Baseline immunological and haematological parameters were performed on the blood samples that were collected from the patients by registered medical personnel before supplementation. Full blood count, $CD4^{+}$T-cell count, $CD8^{+}$T-cell count were determined at screening and monthly visits, while the viral load was done at the screening and at the end of study.

3.6.5.2 Haematological Parameters

A Beckman Coulter machine was used to determine the haematological parameters. The haematological parameters determined in this study include red cell count, haemoglobin, white cell count, differential count, haematocrit (Hct), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW), and platelet count.

(i) Principle of Operation of the Beckman Coulter Machine

The Coulter method counts and sizes cells by detecting and measuring changes in electrical resistance when a particle in a conductive liquid passes through a small aperture. As each cell goes through the aperture, it impedes the current and causes a measurable pulse. The number of pulses signals the number of particles. The height of each pulse is proportional to the volume of that particle.

While the number of pulses indicates particle count, the amplitude of the electrical pulse produced depends on the cell's volume. Theoretical analysis of the behaviour of particles within an aperture shows that the height of the electrical pulse produced by the cell is the characteristic that most nearly exhibits proportionality to the cell volume (Dacie & Lewis, 1985).

(ii) Procedure

(a) White blood cell, Red blood cell and Platelet count

The procedure for determining the haematological parameters was done according to the manufacturer's instruction (Beckman, USA).

(b) Measurement of Haemoglobin

The system used the lysed WBC dilution to measure haemoglobin. The absorbance of light from a Light Emitting Diode (LED) is measured at 525 nm through the optical pathlength of the bath. A beam of light from an LED passes through the sample, through a 525 nm filter and is measured by a photodiode. The signal is amplified and the voltage is measured and compared to the blank reference reading.

(iii) Derivation of Parameters

(a) White blood cell (WBC) counts

This is the number of leucocytes measured directly, multiplied by a calibration constant and expressed in thousands of leucocytes per microlitre of whole blood.
$WBC = n \times 10^3/\mu l$

(b) Red blood cell (RBC) counts

This is the number of erythrocytes measured directly, multiplied by a calibration constant and expressed in millions of erythrocytes per microlitre of whole blood
$RBC = n \times 10^6/\mu l$

(c) Platelet count

This is the number of thrombocytes derived from the platelet fitted curve, multiplied by a calibration constant and expressed in thousands of thrombocytes per microlitre of whole blood.

Platelet = n x 10^3 cells/µl.

(d) Mean cell volume (MCV)

This is the average volume of individual erythrocytes derived from the RBC histogram. The system multiplies the number of RBC in each channel by the size of the RBC in that channel. The products of all channels between 36 fl and 360 fl are added. The analyser then multiplies the count by a calibration constant and expresses MCV in femtolitres (fl).

(e) Haematocrit (Hct)

This is the computed relative volume of erythrocytes and is expressed as a percentage.

Haematocrit (%) = RBC x MCV/10

(f) Mean cell haemoglobin (MCH)

This is the computed weight of haemoglobin in the average erythrocyte, expressed in picograms (pg)

MCH (pg/cell) = Haemoglobin x10/RBC.

(g) Mean cell haemoglobin concentration (MCHC)

This is the computed average weight of haemoglobin in a measured dilution, expressed in grams of haemoglobin oer decilitre (dl) of erythrocytes.

MCHC (g/dl) = Haemoglobin x 100/Haematocrit.

(h) **Red cell distribution width (RDW)**

This is the size distribution spread of the erythrocyte population derived from the RBC histogram. It is the coefficient of variation (CV) expressed as a percentage of the RBC size distribution.

Platelet = n x 10^3 cells/µl.

3.6.5.3 Method for Determining CD4$^+$T-cell Count

(i) **CD4$^+$/CD8$^+$T-cell Counts**

The CD4$^+$/CD8$^+$T-cell counts are measured using a principal method called flow cytometry in which blood cells are passed through a specially designed flow chamber and the physical characteristics of the cells (size and granularity) are measured with laser technology. This process also leads to 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$^+$T-cell counts supplied by Becton Dickinson (BD Ltd, South Africa was used for this study.

(a) **Principle**

When whole blood is added to the reagent, the fluorochrome-labelled antibodies in the reagent bind specifically to the leucocyte surface antigens. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about the cell's size, internal complexity and relative fluorescence intensity. Multi TEST reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population to reduce contamination of unlysed or nucleated red blood cells in the gate. The Multi TEST program on the FACSCalibur flow cytometer automatically gates the lymphocyte population from a CD45 against side scatter display (Jackson, 1990).

Human lymphocytes can be divided into three major populations based on their biological function and cell-surface antigen expression: T-lymphocytes, B-lymphocytes and natural killer (NK) lymphocytes. BD Multi TEST CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC is used to identify and determine the percentages and absolute counts of mature human T-lymphocytes ($CD3^+$), suppressor/cytotoxic T-lymphocyte subsets ($CD3^+/CD8^+$) and helper/inducer T-lymphocyte subsets ($CD3^+/CD4^+$) in an erythrocyte-lysed blood sample (Jackson, 1990; Romeu *et al.*, 1992; Martin, 2000).

(b) Procedure for Measuring $CD4^+/CD8^+$T cell Count

The EDTA tube containing a patient's blood sample was vortexed for a few seconds to allow for proper mixing of the sample. Each sample was checked for clots with an applicator stick and the applicator stick was subsequently disposed of. Ten (10) µl (1:1 diluted) of CD3/CD4/CD45 monoclonal antibody was placed into each tube. Fifty (50) µl of the patient's whole blood was added to the tube containing the antiserum. The same was done with the Immuno-Trol control. The tube was vortexed and incubated in the dark at room temperature for 15 minutes. Five hundred (500) µl of lysing solution was added to the mixture of antibody and blood and spun on a vortex machine. The tube was further incubated in the dark at room temperature for 15 minutes. The specimen mixture was then analysed on the FACS Calibur MULTISET programme

3.6.5.4 Determination of Viral Load (HIV-RNA)

The Amplicor HIV-1 Monitor Test, version 1.5 (v 1.5) was used for the quantitation of HIV-RNA in this study (Roche Diagnostic Systems, Inc., USA). It is an *in vitro* nucleic acid amplification test for the quantitation of HIV-1 in human plasma.

(i) Sample Collection

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

(ii) Separation of Blood Samples

The plasma was separated from the whole blood by centrifugation at 800 revolutions per minute for 20 minutes at room temperature. The separation occurred within 6 hours of collection. Plasma specimens were stored frozen at -80^0C and analysed in due course.

(iii) Specimen Preparation

The AMPLICOR HIV-1 MONITOR Test, v 1.5, can be used with either of two specimen preparation procedures, viz the Standard procedure or the Ultra Sensitive procedure. For this study, the standard procedure was used. 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.

(iv) Principle of the Test

The Amplicor HIV-1 Monitor Test, v 1.5, is based on five major processes: specimen preparation; reverse transcription of target RNA to generate complementary DNA (cDNA); PCR amplification of target cDNA using HIV-1 specific complementary primers; hybridization of amplified DNA to oligonucleic probes specific to the targets, and 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 quantitation of HIV-1 viral RNA is performed using the HIV-1 Quantitation 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 identical primer binding sites to the HIV RNA target and a unique probe binding region that allows quantitation standard amplicon to be distinquished from HIV-1 amplicon. The Quantitation Standard is 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 is amplified together with the HIV-1 target. HIV-1 RNA levels in the test specimens are determined by comparing the HIV-1 signal to the quantitation standard signal for each specimen. The quantitation standard compensates for effects of inhibition and controls the amplification process to allow the accurate quantitation of HIV-1 RNA in each specimen (Myers & Gelfand, 1991; Wilson, 1990; Kwok & Sninsky, 1993; Saag *et al*., 1996).

3.6.6 Physical Examination

A general physical examination was performed during the screening and monthly visit by a medical practitioner (Appendix D). All concomitant medications given to patients during the course of study were documented in the clinical reference form (CRF).

3.7 Statistical Analysis

The results obtained from this study were analysed by an independent Biostatistician at the University of the Free State, South Africa. For all the patients as well as for genders separately, the following statistical analysis was done for the monthly, screening and final visits.

Continuous variables were described by using mean, standard deviation and median or percentiles where applicable. Categorical variables were described by frequencies and percentages. Some variables were classified into categories (normal values, less than normal or greater than normal values) and were described by frequencies and percentages, together with other categorical variables.

The difference between screening visit and final visit (screening-final) was calculated for continuous variables and described by medians and percentiles and the results were compared using the Willcoxon Igned Rank test as well as non-parametric confidence intervals for the median paired difference.

The change in categories of the variables, classified according to normal ranges from screening visit to final visit, was described by contingency tables.

The difference between the genders during each of the screening and final visits was calculated and the genders were compared using the Chi-Square test for categorical variables. Continuous variables were compared using the Mann-Whitney test and 95% non-parametric confidence intervals.

Spearman rank order correlations were calculated between some of the variables. The analysis of variables was done using SAS (1990).

3.8 Good Clinical Practice (GCP)/Quality Assurance

The study was conducted in compliance with the protocol and the following recommendations and guidelines:

- Department of Health, South Africa (Clinical Trials Guidelines, 2000)
- The declaration of Helsinki (Scotland Revision, 2002)
- ICH Guideline for Good Clinical Practice (1997)

Internal checking was carried out at all stages of the study.

3.9 Summary

This was a clinical trial to determine the influence of a nutritional supplement on the immune, haematological parameters, anthropometric profile and clinical conditions of HIV-positive/AIDS patients.

The socio-demographic composition of the patients was determined using a questionnaire. A validated food frequency questionnaire was used to assess the baseline macronutrient and micronutrient intakes of the patients. Weight, height, circumference (waist and hip) and skinfold measurements were obtained and employed to calculate the body mass index, fat distribution and percentage fat of patients respectively. Assessment of the physical and medical conditions of the patients was done by a medical practioner and the information entered into a clinical reference form (CRF).

At baseline, the socio-demographic and food frequency questionnaires were completed. The anthropometric measurements were performed at baseline as well as at the end of nutrient supplementation. Blood samples were collected by health personnel for haematological parameters, immune factors and viral load and determined by standard methods and procedures. The haematological parameters, immune factors were repeatedly monthly while the viral load was repeated at the end of the study.

The statistical analyses (mean, median, percentiles, differences and correlations) of the variables was done by an independent Biostatistician at the Faculty of Health sciences, University of the Free State.

3.10 Limitations of the study

Certain factors limited the outcome of the results of this study and stated below:
In general, the cross-sectional nature of the study, the small number of patients studied, the absence of a control group and the lack of references adapted for HIV-positive/AIDS patients, make it difficult to extrapolate the results of this study.

Furthermore, in the present study, there is a lack of information on some important predictors of patients' nutritional status, for instance, blood levels of the corresponding nutrients analysed using food frequency questionnaire in the study were not determined and therefore prevent one from making casual references or definite conclusions

Although necessary precautions were taken in collating dietary data from patients (a food frequency questionnaire was used to determine the habitual types, quantities and frequency of food and drink consumption by recording consumption on a daily, weekly and monthly basis over a period of six months prior to the study), it is possible that difficulties regarding recalling food intake correctly and over reporting were experienced and possibly contributed to the dietary intake recorded. Also, whether the increase in energy intake is sustained every day or is only representative of their intake during best days or days during during recording of food intake, cannot be confirmed by the food frequency questionnaire at baseline.

It is possible that certain factors such as malabsorption, and drug-nutrient interaction (for example, TB treatment) could have limited the potential positive effect of the supplement on the parameters measured.

Time of collection and analysis of blood samples was a factor that limited the outcome of the study.

3.11 References

Allard JP, Aghdassi E, Chau J, Tam C, Kovacs CM, Salit IE *et al* (1998). Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. **AIDS 12**: 1653-1659.

Carter JEL & Heath B (1990). Somatotyping-development and applications. Cambridge: Cambridge University Press; 66-73.

Castetbon K, Kadio A, Bondurand A, Boka Yao A, Barouan C (1997). Nutritional status and dietary intakes in human immunodeficiency virus (HIV)-infected outpatients in Abidjan, Cote d' Ivoire, 1995. **European J Clin Nutr 51**: 81-86.

Coodley GO, Nelson HD & Loveless MO (1993). Beta-carotene in HIV infection. **J Acquir Immune Def Syndr 6**: 272-276.

Dacie JV & Lewis SM (1985). Practical Haematology. 6th Edition. ChurchHill Livingstone: Medical Division of Longman Group Ltd, New York; 68-79.

Dannhauser A, Van Staden AM, Van der Ryst E, Nel M, Marais N, Erasmus E *et al* (1999). Nutritional status of HIV-1 sero-positive patients in the Free State Province of South Africa: Anthropometric and dietary profile. **European J Clin Nutr 53**: 165-173.

Department of Health South Africa (2000). Clinical trials guidelines: 1-50.

Dwyer JT (1998). Dietary assessment. In: Modern nutrition in health and disease. ME Shils, JA Olson, M Shike & AC Ross (eds) 9th Edition. Philadelphia, Lea & Febiger: 942.

Food & Nutrition Board (1989). Recommended Dietary Allowances. 10th ed. Washington, DC: National Academy Press: 5-31.

Food & Nutrition Board (2001). In e DRI: dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington, DC: National Academy Press: 5-78.

Gibson R (1998). Determining nutritional status. In: Mann J & Truswell AS (ed). Essentials of Human Nutrition. 1st Edition, Oxford University Press: 398-405.

Hammond KA (2004). Dietary and Clinical Assessment. In: Mahan LK & Escott-Stump S (eds). Krause's Food, Nutrition and Diet Therapy. 11th Edition Philadelphia: WB Saunders Co: 372.

Heath BH & Carter JEL (1967). A modified somatotype method. **Am J Physical Anthropology 27**: 57-74.

Helsinki (2000). A Revised Declaration of Helsinki, Edingburgh, Scotland Dec 18: 1-56.

ICH Guidelines (1997). ICH guidelines for good clinical practice 17 January: 10-22.

Jackson AS & Pollock ML (1985). Practical assessment of body composition. **Physician Sport Med 13**: 76-90.

Jackson A (1990). Basic phenotyping of lymphocytes: selection and testing of reagents and interpretation of data. **Clin Immunol Newslett 10** (4): 49-55.

Japour AJ (1996). Measurement of HIV-1 RNA in clinical practice: an initial management algorithm. **J Int Assoc of Physicians in AIDS Care 2**: 16-19.

Kwok S & Sninsky JJ (1993). PCR detection of human immunodeficiency virus type-1 proviral DNA sequences. In: D.H Persing, T.F Smith, F.C Smith, T.J Tenover & T.J White (eds). Diagnostic Molecular Biology: Principles and Applications. ASM Press, Washington, DC: 1-27.

Langenhoven ML, Kruger M, Gouv (1998). Medical Research Council (MRC) Food Composition Tables . 4th Edition. Parow, South Africa: National programme: Nutritional Intervention Medical Research Council.

Laquatra I (2004). Nutrition for weight management. In: Mahan LK & Escott-Stump, S (ed). Krause's Food. Nutrition & Diet Therapy. 10th Edition. Philadelphia: WB Saunders company: 488-493

Lee RD & Nieman DC (1996). Nutritional Assessment. 2nd Edition. McGraw Hill companies: 224-272.

Martin D (2000). Appropriate laboratory monitoring of HIV. **S Afri Med J 90** (1): 33-35.

Mulder J, McKinney N, Christopherson C (1996). Rapid and simple PCR assay for quantitation of HIV-1 RNA in plasma: Application to acute retroviral infection. **J Clin Microbiol 32**: 292-300.

Myers TW & Gelfand DH (1991). Reverse transcription and DNA amplification by a Thermus thermophilus DNA polymerase. **Biochemistry 30**: 7661-7666.

Niyongabo T, Bouchaud O, Henzel D, Melchior JC, Samb B, Dazza MC *et al*. (1997). Nutritional status of adult inpatients in Bujumbura, Burundi (impact of HIV infection). **European J Clin Nutr 51**: 637-640.

Paton NIJ, Macallan DC, Jebb SA, Noble C, Baldwin C, Pazianas M *et al*. (1996). A comparison of methods for measuring changes in fat-free mass in patients with acquired immune deficiency syndrome. **Proc Nutr Soc 56**: 153A.

Pressman AH & Adams AH (1990). Clinical assessment of nutritional status: A working manual. 2nd Edition. Baltimore: Williams & Wilkins: 52.

Romeu MA, Mestre M, Gonzalez L (1992). Lymphocyte immunophenotyping by flow cytometry in normal adults: comparison of fresh whole blood lysis technique, Ficoll-Paque separation and cytopreservation. **J Immunol Methods 154**: 7-10.

Saag MS, Holodniy M, Kuritzkes D [illegible] ral load markers in clinical practice. **Nature Med 2**: 625-629.

Sappey C, Leclercq P, Coudray C, Faure P, Micoud M & Favier A (1994). Vitamin, trace element and peroxide status in HIV sero-positive patients: asymptomatic patients present a severe beta-carotene deficiency. **Clinica Chimica Acta 230**: 35-42.

SAS (1990). SAS Procedures Guide, version 6. 3rd Edition, Cary NC: SAS Institute Inc: 991-10705.

Schmidt PK & Carter JEL (1990). Static and dynamic differences among five types of skinfold callipers. **Human Biology 62**: 369-388.

Smolin LA & Grosvenor MB (2000). Nutrition science and applications. 3rd Edition. Saunders College Publishing, Philadelphia pp 213.

Twigg HL, Spain BA, Soliman DM, Knox K, Sidner, Schnizlein-Bick *et al.* (1999). Production of interferon gamma by lung lymphocytes in HIV-infected individuals. **Am J Physiol Lung & Cell Mol Physiol 276**: L256-L262.

Van Staden AM, Barnard HC, Nel M, Attwood EM, Dannhauser A, Van der Ryst E *et al.* (1998). Nutritional status of HIV-1 sero-positive patients in the Free State Province of South Africa-laboratory parameters. **Cent African J Med 44** (10): 246-250.

Willett WC (1990). Nutritional Epidemiology. New York: Oxford University Press: 34-41.

Wilson CB (1990). The cellular immune system and its role in host defence. New York, NY: Churchill Lvingstone Inc: 101-138.

Chapter 4

An Analysis of the Baseline Dietary Intake of HIV-positive/AIDS Patients Residing in a Low Socio-economic Community in Bloemfontein

4.1 Abstract

This study analysed the baseline dietary intake of people living with HIV/AIDS in the African community of Bloemfontein. A validated food frequency questionnaire was used to assess the dietary intake of both macronutrients and micronutrients in 35 HIV-positive/AIDS patients. The patients demonstrated energy and dietary intake of major macronutrients higher than the EEI and RDA respectively and this tended to be higher ($P<0.05$) in males than in females. The result also showed that the mineral and trace element dietary intake exceeded the RDA/AI, except for iodine and selenium. The majority of the patients reported adequate intake of most vitamins; however a relatively high percentage of the patients indicated an inadequate intake for folate and vitamin D. It is envisaged that the high dietary intake of major macronutrients and micronutrients will help in maintaining the nutritional status and in reducing wasting in the patients. However, the relatively high percentage of the patients with an inadequate intake of iodine, selenium, folate and vitamin D is of great concern and calls for urgent attention.

4.2 Introduction

Good nutrition is the cornerstone for maintaining an optimum immune response (Woods *et al.,* 2002). Normal antibody production, phagocytic cell and T-lymphocyte functions depend on the adequate intake of energy, protein, fat, minerals and vitamins (Semba, 1994; Scrimshaw & SanGiovanni, 1997). It is a fact that malnutrition alters the immune function with a subsequent increase in susceptibility to infections (Semba & Tang, 1999), faster disease progression (Scrimshaw & SanGiovanni, 1997; Baum & Shor-Posner, 1998; Macallan, 1999), reduced functional status, quality of life, and increased morbidity and mortality (Scrimshaw & SanGiovanni, 1997; Kotler, 1997).

Nutritional deficiencies are well-documented complications of HIV infection (McCorkindale *et al*., 1990; Cuff, 1990; Macallan, 1999). HIV-positive/AIDS patients develop severe protein and energy malnutrition. Several vitamins and minerals have been found to be deficient as well. The pathogenesis of nutritional impairment in HIV-positive patients is multifaceted and includes decreased food intake, decreased nutrient absorption and decreased efficiency of utilisation, in addition to increased nutritional demand (Abrams *et al*., 1993; Baum *et al*., 1995; Castetbon *et al*., 1997; Piwoz & Preble, 2000). Literature reports (Fawzi & Hunter, 1998; Kupka & Fawzi, 2002) clearly indicate that HIV infection contributes to malnutrition for physiological reasons related to HIV infection itself and because most people living with HIV/AIDS, particularly in developing countries, often have diets that are deficient in energy, proteins, vitamins and other nutrients. Friis and Michaelsen (1998) reported that macro-and micronutrient deficiencies could impair host immune functions and affect viral replication and pathogenicity, thus potentially affecting the clinical course of HIV infection. Therefore, nutrition may play a role in the progression of HIV infection to AIDS, as well as mortality from AIDS. Other studies indicate that in the early period of HIV infection, weight gain or maintenance might be achieved through adequate diet and that this has helped to reduce the consequences of wasting in people living with HIV/AIDS (Semba & Tang, 1999; Piwoz & Preble, 2000).

Chlebowski *et al.* (1989) reported reduced dietary intake in AIDS patients compared with asymptomatic HIV-positive subjects. McCorkindale *et al.* (1990) demonstrated decreased food intake even in asymptomatic HIV-infected subjects and early AIDS Related Complex (ARC) subjects during a 16-month period, and this was associated with significant weight loss. On the other hand, Dworkin *et al.* (1989) and Kotler *et al.* (1990) found no differences in dietary intake among patients with AIDS or AIDS-Related Complex (ARC) and asymptomatic HIV-positive controls or uninfected controls. Hellerstein *et al.* (1990) found that dietary intake was adequate in most HIV-positive/AIDS persons.

Research findings by Tindall & Cooper (1991) and Allard *et al.* (1998) showed that up to 50-90% of individuals infected with HIV develop symptomatic primary HIV infection, within couple of weeks of being infected with the HIV. In addition to the metabolic effects of the febrile illness associated with HIV infection, some specific signs and symptoms of primary HIV infection may contribute to the impairment of nutritional status. It is reported that with the progression of HIV infection to an advanced stage and in addition to fever, opportunistic infections or malignar [illegible] duced food intake due to reduced appetite or taste disturbances, such as painful mucosa and lesion of the mouth, pharynx or oesophagus (Tindall & Cooper, 1991). Likewise, 30-50% of patients in developed countries and about 90% in developing countries developed diarrhoea and hence malabsorption. These factors contribute to impaired nutritional status in HIV-positive/AIDS patients (Smith *et al.,* 1992; Macallan, 1999), further affecting the nutritional status of people living with the infection/disease. Besides this, economic factors, in particular poverty (Babamento & Kotler, 1997) and limited food preparation facilities may limit the HIV-positive patients' ability to prepare and have access to foods (Cimoch, 1997), thus influencing food and nutrient intake haphazardly. It has also been shown that emotional instabilities such as depression, isolation (as a result of social discrimination), inaccessibility to and unavailability of food, and difficulties patients have in caring for themselves in the late stage of the disease can further affect and reduce food intake (Grunfeld & Feingold, 1992; USAID, 2001; Department of Health, South Africa, 2002).

The effects of HIV infection on the nutritional status are likely to be more pronounced among under-privileged populations with a low dietary intake. However, only few data have been reported in developing countries (Semba *et al.,* 1994; Phuapradit *et al.*, 1996; Castetbon *et al.*, Dannhauser *et al.*, 1999). Baum *et al*. (1995) concluded that although the metabolic response to HIV infection may contribute to low nutritional status in HIV-infected individuals, there is no doubt that the intake of nutrients at levels recommended for the general population does not appear to be adequate for HIV-1 infected persons. Thus, the importance of any possible effects of nutritional deficiency on HIV progression is evident.

Malnutrition associated with HIV infection has serious and direct implications on the quality of life of people living with HIV and AIDS (Piwoz & Preble, 2000). Weight loss is often the early symptom, followed by a vicious cycle of increased fatigue and decreased physical activity, including the inability to prepare and consume food (Semba & Tang, 1999). Malnutrition associated with HIV/AIDS affects entire families when infected adults become too debilitated to work steadily, unable to provide for themselves and their dependants and require continual care during bouts of illness (Piwoz & Preble, 2000).

An association between physiologic[illegible]encies and disease progression has been demonstrated in developed countries (Baum *et al*., 1995), while a possible relationship between dietary intake and disease progression has also been established (Dworkin *et al*., 1990). However, there are limited data in reference to dietary intakes and disease progression in HIV-infected persons from African countries and further studies in African countries have been advocated (Castebon *et al*., 1997; Dannhauser *et al*., 1999).

The aim of this study was to assess the nutritional status by analysing the dietary intake and its association with the stage of HIV infection among HIV-positive/AIDS patients living in a low socio-economic community in Bloemfontein, South Africa.

4.3 Subjects and Methods

A cross-sectional descriptive study was carried out at Medi Inn Clinic, Bloemfontein and it formed part of a clinical trial that involved a target group (HIV-positive/AIDS patients). Fifty HIV-1 sero-positive/AIDS patients were recruited from Tsepo home-based care and from low socio-economic community in Bloemfontein with the assistance of the Red Cross home-based care. An approval from the Ethics Committee of the Faculty of Health Sciences, University of the Free State was obtained and all patients gave informed consent for inclusion in the study. See chapter 3 for details (see **3.4**).

4.3.1 Sample collection

This is described in chapter 3 under methodology (see **3.5.5.1**)

4.3.2 Dietary intake

The detailed information is available in chapter 3 under methodology (see **3.5.3**)

4.3.3 Statistical analysis

The statistical analyses of results was done by an independent Biostatistician at the Faculty of Health Sciences, University of the Free State, South Africa. The details are described in chapter 3 (see **3.5.7**).

4.4 Results

A total of 50 HIV-positive/AIDS volunteers were enrolled in this study after screening. Three patients dropped out, 10 died and 2 relocated to other towns during the period of the trial. Eight (8) of the 35 patients who completed the food frequency questionnaire were male (22.9%) while 27 (77.1%) were female.

Table 4.1: Baseline energy, maj[illegible]ts and cholesterol intake of HIV-positive/AIDS patients.

Nutrients	Mean	SD	Median	RDA/AI	% of patients <67% of RDA/AI
Energy (KJ)/day					
Male n=8	20217.4	6992.8	19316.7	12180	0
Female n=27	18402.8	9102.2	17266.5	9240	11.1
Whole Group n=35	18817.6	8603.5	17889.6	10710	8.6
Plant protein (g)/day					
Male	64.8	29.0	56.8		0
Female	64.0	35.7	62.1		0
Whole Group	62.3	33.9	62.1		0
Animal protein (g)/day					
Male	86.2	24.7	92.9		0
Female	91.5	79.9	75.3		0
Whole Group	90.3	70.8	84.6		0
Total protein (g)/day					
Male	155.9	48.0	131.3	63	0
Female	159.8	98.6	131.3	50	3.7
Whole Group	158.9	89.0	131.7	56.5	2.9
Total CHO (g)/day					
Male	605.8	208.5	554.4	358-430	0
Female	573.8	315.6	548.2	272-324	3.7
Whole Group	581.2	292.1	548.2	298-346	2.9
Total dietary fibre (g)/day					
Male	40.5	14.1	37.2	38*	12.5
Female	43.8	26.4	43.6	25*	14.8
Whole Group	43.1	24.0	46.6	31.5*	14.3
Total fat (g)/day					
Male	175.3	75.7	149.4	<96	0
Female	140.0	65.8	132.9	<73	0
Whole Group	148.1	68.7	144.7	<84.5	0
Cholesterol (mg)/day.					
Male	595.2	222.0	592.0	<300	0
Female	536.5	522.4	382.1	<300	0
Whole Group	549.9	468.5	437.5	<300	0

*AI (Adequate intake)

The nutrient intakes with their m eviation, median, RDA/AI and the percentage of patients with a dietary intake of less than 67% of the RDA/AI, are presented in table 4.1. The male (n=8) and female (n=27) HIV-positive/AIDS patients examined in this study demonstrated energy and dietary intakes of major macronutrients higher than the EER and RDA values respectively. Observation of the results showed that the dietary intake tends to be higher in males ($P<0.05$) than in females except for animal and plant protein which were higher in females. As whole group, the majority of the patients reported an adequate dietary intake.

Table 4.2: Baseline mineral and tr[illegible]ke of HIV-positive/AIDS patients.

Nutrients	Mean	SD	Median	RDA/AI	% of patients <67% of RDA/AI
Calcium (mg)/day					
Male n=8	1637.0	816.7	1381.1	1200*	0
Female n=27	1257.1	618.8	1281.9	1000*	18.5
Whole Group n=35	1343.9	675.5	1295.7	1100*	14.3
Copper (mg)/day					
Male	3.1	1.8	2.2	0.9	0
Female	3.4	3.9	2.5	0.9	7.4
Whole Group	3.4	3.5	2.5	0.9	5.7
Total iron (mg)/day					
Male	25.0	13.8	19.0	8	0
Female	25.6	19.3	22.2	18	25.9
Whole Group	25.4	18.0	21.4	13	20
Chromium (mg)/day					
Male	70.9	30.3	57.9	25*	0
Female	61.0	58.0	42.8	25*	11.1
Whole Group	63.2	52.7	52.7	25*	8.6
Zinc (mg)/day					
Male	20.4	7.3	20.3	11	0
Female	18.1	10.4	16.3	8	7.4
Whole Group	18.6	9.7	18.0	9.5	5.7
Magnesium (mg)/day					
Male	706.4	268.7	646.6	420	0
Female	696.8	353.7	674.4	320	7.4
Whole Group	699.0	332.4	674.4	370	5.7
Selenium (mg)/day					
Male	77.0	37.0	71.5	55	12.5
Female	71.1	78.8	38.6	55	48.2
Whole Group	72.4	71.0	45.4	55	40
Iodine (mg)/day					
Male	74.0	27.8	78.3	150	75.0
Female	52.9	41.4	38.8	150	82.9
Whole Group	57.7	39.4	46.7	150	82.9

*Adequate Intake

An examination of the mineral and [illegible] akes as shown in table 4.2, revealed that the median intake exceeded the RDA/AI, except for iodine. In the male patients (n=8), 12.5% took in less than 67% of the RDA for selenium while 75% of the male patients took in less than 67% for iodine. On the other hand, 48.2% of the female patients (n=27) had an inadequate intake of selenium and 82.9% of the female patients (n=27) had an inadequate intake of iodine. The micronutrient intakes were also higher in the male patients than in the female patients with the exception of copper and total iron. This inadequate intake of selenium and iodine is also reflected in the whole group of patients studied. The difference in the number of male and female patients was taken into consideration in the statistical analysis of the data.

Table 4.3: Baseline vitamin intake [illegible] AIDS patients.

Vitamins	Mean	SD	Median	RDA/AI	% of patients < 67% RDA/AI
Niacin (mg NE)/day					
Male n=8	38.5	20.6	29.3	19	0
Female n=27	36.1	25.8	29.9	14	7.4
Whole Group n=35	36.7	24.4	29.9	11.5	5.7
Riboflavin (mg)/day					
Male	3.7	1.9	3.1	1.3	0
Female	3.1	3.3	2.5	1.1	7.4
Whole group	3.2	3.0	2.7	1.2	5.7
Thiamine (mg)/day					
Male	3.3	1.9	2.6	1.2	0
Female	3.4	2.3	2.7	1.1	7.4
Whole Group	3.3	2.2	2.7	1.2	5.7
Vitamin A (μg RE)/day					
Male	1485.9	1010.7	1171.6	900	12.5
Female	2179.3	2879.7	1221.9	700	14.8
Whole Group	2020.8	2576.6	1221.9	800	14.3
Vitamin E (mg α TE)/day					
Male	24.03	9.92	21.3	15	0
Female	23.85	15.89	17.0	15	14.8
Whole group	23.89	14.61	20.4	15	11.4
Vitamin C (mg)/day					
Male	199.0	213.2	137.6	90	12.5
Female	223.7	225.6	139.4	75	18.5
Whole group	218.1	220.0	139.4	82.5	17.1
Vitamin D (μg)/day					
Male	10.3	6.00	9.4	15*	12.5
Female	6.2	4.5	5.0	5*	29.6
Whole Group	7.2	5.1	5.8	10*	25.7
Vitamin K mg/day					
Male	199.5	161.2	141.8	120*	25.0
Female	287.0	405.1	134.9	90*	11.1
Whole Group	267.0	363.7	134.9	105*	14.3

*Adequate Intake

Table 4.3 (continued): Baseline vi IV-positive/AIDS Patients.

Vitamins	Mean	SD	Median	RDA/AI	% of patients <67% RDA
Vitamin B_6 (mg)/day					
Male	3.4	1.5	3.0	2.4	0
Female	3.0	1.7	2.6	1.3	7.4
Whole group	3.1	1.6	2.6	1.4	5.7
Vitamin B_{12} (μg)/day					
Male	16.8	15.2	10.2	1.7	0
Female	23.6	47.8	10.7	2.4	0
Whole group	22.1	42.4	10.4	2.5	0
Folate (μg)/day					
Male	406.7	280.8	287.4	400	37.5
Female	399.4	300.3	332.7	400	33.3
Whole group	401.1	291.9	332.1	400	34.3

*Adequate Intake

Table 4.3 shows that the majority of the male and female patients and the whole group demonstrated median dietary intake higher than the RDA/AI. In addition, table 4.3 indicates the percentage of patients who took in less than 67% of the RDA/AI for these groups of patients. The median dietary intake of vitamins A, C, K, folate and B_{12} was higher in the female patients than in the male patients. The male group reported a higher intake of other vitamins except riboflavin and thiamine. A relatively high percentage of the study population reported an inadequate intake of folate and vitamin D

4.5 Discussion

Adequate nutrition, which is best achieved through consumption of a balanced healthy diet is vital for the health and survival of all HIV-infected persons. Energy requirements are likely to increase by 10% to maintain body weight and physical activity in asymptomatic HIV-infected adults. During symptomatic HIV infection, and subsequently during AIDS, energy requirements increased by approximately 20% to 30% to maintain adult body weight (WHO, 2003).

As observed in this study, the medi is higher than the EER for both male and female patients (table 4.1). This agrees with previous findings as reported by Hogg *et al.* (1995) that HIV-infected patients had a higher energy intake than their HIV-negative counterparts. The present results also confirm the World Health Organization (WHO, 2003) report that energy requirements increase significantly during the symptomatic stage of HIV-infection. It is important to note that energy intake is related to the stage of the infection, rapid weight loss, anorexia, opportunistic infections, malabsorption and altered metabolism (Kotler *et al.*, 1990). In another study performed by Kim *et al.* (2001), it was suggested that energy and dietary intake is a complex socio-behavioural phenomenon that reflects the confluence of attitudinal, economic and lifestyle factors. In the population studied, these factors may have contributed in one way or the other to the higher energy intake. Although necessary precautions were taken in collating dietary data from patients (a food frequency questionnaire was used to determine the habitual types, quantities and frequency of food and drink consumption by recording consumption on a daily, weekly and monthly basis over a period of six months prior to the study), it is possible however that difficulties regarding recalling food intake correctly and over reporting were experienced and possibly contributed to the dietary intake recorded.

It is also possible that the higher energy intake may be directly or indirectly related to the staple food consumed by the population. Whether this increase in energy intake is sustained every day or is only representative of their intake during "best" days or days during recording of food intake, cannot be confirmed by the food frequency questionnaire at baseline. If it is agreed with the WHO (2003) that energy requirements increase significantly as the HIV disease progresses, then it might be viewed as a good trend for patients with a high energy intake as reported in this study. The higher energy intake could assist to a certain degree in reducing wasting and improve the well-being of the patients. According to Macallan (1999), reduced energy intake promotes wasting.

Both male and female patients reported a median total protein intake of 131.3 g higher than the RDA of 63 g and 50 g respectively, while 3.7% of the female patients took in less than 67% of the RDA (table 4.1). Walsh *et al.* (2003, unpublished work) also reported a median total protein intake in HIV-positive patients that was higher than the RDA. According to Dannhauser *et al.* (1999), and Kim *et al.* (2001) studies carried out on HIV-infected patients in the Free State province of South Africa and in Boston (USA) respectively, reported that majority of the patients had a total protein intake that met at least 67% of the RDA. The high intake of total protein as observed in this study is suggested to be associated with urbanisation. It is believed that, in some cases, urbanisation is accompanied by an increased intake of animal protein depending on the economic status of the individual. It has been observed that diets become diverse with urbanisation and that more people add meat, fish, diary products. eggs and cheese to their meals (Drewnowski & Popkin, 1997). The availability of cheaper cuts of red meat, offal, sausage, chicken and chicken offal could have contributed to the high intake of total protein in the studied population. The median plant protein intake (62.1 g) in the female subjects was higher than that of their male counterparts [56.8 g] (table 4.1). The median plant protein intake reported for male and female subjects was lower than the animal protein intake for both sexes (being 92.9 g in male and 75.3 g in the female subjects, table 4.1). This is in line with the work of MacIntyre (1998) who found that the ratio of plant to animal protein intake has changed significantly in the diets of urbanised Africans, with rural Africans consuming more plant proteins than their urban counterparts. The high total protein intake may compensate for the increased urinary nitrogen loss, increased protein utility, decreased skeletal protein synthesis and increased skeletal muscle breakdown that is reported in HIV-infected individuals. The differences in plant and animal protein intake between male and female subjects, as seen in this study, is not clear, but may partly be related to economic and social factors.

The median total carbohydrate intake of both male and female subjects exceeded the RDA and may have contributed significantly to the energy intake, required to meet the metabolic needs of HIV-positive/AIDS patients. The median intake of dietary fibre in the male patients was slightly lower than the AI intake, but was not significantly lower than the AI while the median dietary fibre intake of female subjects exceeded the AI (table 4.1). The dietary fibre is known to reduce colonic cancer and the consumption of minimally processed foods such as whole-wheat bread and whole-wheat rice should be encouraged in respect of HIV-positive/AIDS patients. On the whole, the total carbohydrate intake may compensate for increased peripheral glucose utilization and increased gluconeogenesis common in advanced HIV disease (Piwoz & Preble, 2000).

The total fat intake of 149.4 g/day for male patients and 132.9 g/day for female patients (table 4.1) exceeded the RDA of <96 g and <73 g per day for male and female subjects respectively. In both male and female patients the dietary intakes were significantly higher than the RDA, with the male patients showing a higher intake. The high intake of fat may be ascribed to preference for cheaper fatty red meat, eggs, brick margarine, meat drippings and potato crisps. There is no evidence that total fat needs are increased beyond normal requirements as a consequence of HIV infection. To ensure macronutrient intakes at RDA, HIV-positive/AIDS patients are encouraged to consume healthy diets, nevertheless the dietary intake of macronutrients at RDA may not be sufficient to correct nutritional deficiencies in HIV-infected populations. Because weight and lean body mass changes are major indicators of the health status of people living with HIV/AIDS, an optimal intake of kilojoules and protein is vital and, according to Woznicki & D'Alessandro (1997), the intake of calorie-yielding nutrients (carbohydrates, protein and fat) at levels that exceed the RDA is advisable.

The role of micronutrients in immune and infectious disease is well established (WHO, 2003). Observational studies have shown that low blood levels and decreased dietary intakes of some micronutrients are associated with faster HIV disease progression, altered immune function and mortality. However, these studies' methodological limitations preclude definitive conclusions about the relationship between micronutrient dietary intakes and HIV infection. In this study, the majority of patients demonstrated an adequate or high intake of micronutrients that was 67% or higher than the RDA. The study also revealed the percentage of some micronutrients with an intake of less than 67% of the RDA, such as selenium and iodine (table 4.2) and vitamins, such as A, E, D and folate (table 4.3) in the male patients. The female patients showed a wider range of inadequate intake (<67% of the RDA) of calcium, copper, total iron, chromium, zinc, magnesium, selenium and iodine (table 4.2) and of vitamins (A, E, C, D, folate, niacin, riboflavin, thiamine and B_6, table 4.3).

Evidence from cross-sectional epidemiological studies have shown that some minerals/trace elements may be key factors in maintaining health despite human immunodeficiency virus infection and in reducing mortality. For instance, selenium appears important in reducing the virulence of HIV and slowing the disease progression, while a positive association has been noted between the dietary intake of zinc and the $CD4^+$T-cell count of HIV-infected persons (Jariwalla, 1995; Abrams *et al.*, 1993). Values higher than the RDA were identified for calcium, copper, iron, chromium, zinc and magnesium, but not iodine, in male and female patients in this study population. In male patients 75% had inadequate intake of iodine. The reason for the high percentage of inadequate intake of selenium and iodine, as seen in the population is not quite understood since table salt in South Africa is iodated. Each of the mineral/trace elements examined in this study may contribute to the general well-being of HIV-infected persons.

Calcium has been shown to reduce diarrhoea in HIV-positive/AIDS patients (Harriman, 1989). About 50% of HIV-positive/AIDS patients using protease inhibitors have been found to significantly lose calcium from their bones (Nolan *et al.*, 2000). In the present study, the median calcium intake was higher than the RDA. Therefore, the results obtained from this study tend to suggest that patients with a high dietary intake (>RDA), might be able to replace lost calcium, especially those who may be fortunate to undergo antiretroviral therapy and perhaps reduce the burden of diarrhoea. There is a plan by the South African government to start providing antiretroviral drugs for HIV-infected persons. If this materialises, it is likely that an adequate dietary intake of calcium could play a compensatory role in correcting drug-induced calcium lost from the body.

Copper is a mineral essential in small amount for proper health and required for red and white blood cell maturation, iron transport, cholesterol metabolism, glucose metabolism, immune function and protection against oxidative stress (Bowers, 2002). A recent study showed that a diet deficient in copper affects the immune system (Bowers, 2002) while adequate dietary intake was associated with a significant decrease in the risks of AIDS (Bogden *et al.*, 1990; Tang *et al.*, 1993). In the current study, majority of the patients showed adequate intake of copper with only 5.7% of intake less than 67% of RDA. It is believed that adequate copper intake would enhance immune function and improves the nutritional status of the patients living with HIV/AIDS. It is therefore suggested that HIV-positive/AIDS patients should include legumes, liver, kidney and cow's milk as they are rich source of copper.

Iron is essential for the formation and functioning of red blood cells, and vitamin C is known to promote the absorption of iron. In both male and female patients the median total iron intake was higher than the RDA, nonetheless 25.9% of the female (table 4.2) population took in less than 67% of the RDA. Iron has been observed as a micronutrient that is commonly deficient in HIV infection however, an oversupply of iron is also regarded as harmful in HIV infection. When oversupplied, iron can stimulate free radical production and further damage the immune system (Cairns, 2001). In one study, iron levels were higher in people with HIV infection (Cairns, 2001). Iron blood level was not determined in this study so as to verify its correlation with dietary intake. Considering the danger of an oversupply of iron, it would be appropriate to measure and monitor blood levels of iron so as to be able to regulate its intake in HIV-positive/AIDS patients. As mentioned earlier, 25.9% of the female population had an inadequate intake of iron as opposed to none in the males. The reason for this discrepancy is not clear, but it may be related to the fact that iron is lacking in women's food due to a lack of knowledge, or because of extra demands during the menstrual cycle (women need extra iron until they pass the menopause stage). Anaemia has been associated with increased rates of mortality in American (Sullivan *et al.*, 1998), European (Mocroft *et al.*, 1999) and Malawian (Semba *et al.*, 2001) studies of HIV-infected persons. This may have serious implications for patients in this study with an inadequate intake of iron.

Chromium is mainly involved in the metabolism of glucose but recent research in animal models showed that chromium can enhance the ability of the white blood cells to respond to infection (Hoffman, 1998). Results of the present study indicated a high intake in both males and females except for 11.11% of the female (table 4.2) population with an inadequate intake. Information on the role of chromium in HIV information is virtually unavailable but it is assumed that in its normal biological function, it would help in proper carbohydrate metabolism in HIV-infected persons, as a carbohydrate abnormal metabolism has been reported in HIV-infected persons (Piwoz & Preble, 2000). As observed in this study, the median intake for chromium was higher than the RDA. it is believed that the patients examined in this study will benefit from such high intake.

Another important element that was considered in the analysis is zinc. Zinc is a mineral that is necessary for protein and energy metabolism, as well as in DNA and RNA synthesis. It also appears to be essential for T-cell differentiation and maturation as well as lymphocyte activation (Chan & Collins, 1997; Kupka & Fawzi, 2002). Dietary intakes of zinc in both male and female patients were higher than the RDA. An intake of less than 67% of the RDA was recorded in 7.4% of the females (table 4.2). There is some evidence to an indicate that inadequate dietary intake may contribute to the prevalence of altered nutritional status in HIV-infected individuals, underscoring the importance of maintaining adequate nutritional support for HIV-positive/AIDS patients. Kupka & Fawzi, (2002) found a positive association between dietary intake of zinc and immune status. By deduction, an adequate dietary intake of zinc (other micronutrients inclusive), as seen in the study population, could contribute to maintaining the nutritional and immune status of HIV-positive/AIDS patients. If this applies to other anti-oxidants such as selenium, vitamin A, C and E, this could help in maintaining the oxidative status and in turn in reducing the oxidative stress which is common in HIV-positive/AIDS patients. Oxidative stress in HIV disease may be related to a failure in such anti-oxidant defence mechanisms, as well as to the chronic and progressive inflammatory reaction associated with the development of HIV infection (Malvy *et al*., 1994). It is possible that such a failure in antioxidant defence mechanisms is partly the result of an inadequate intake of these antioxidants (zinc inclusive) in the diets of HIV-positive/AIDS patients either because of the unavailability of the fruits/sources containing the antioxidant or because of economic or social factors.

Magnesium, one of the minerals examined in the present study is needed for energy production and protein synthesis and is found in whole grains, legumes, leafy green vegetables and nuts. An adequate intake can assist in preventing muscle spasms and tremor and it seems likely that an adequate or high intake in HIV-positive/AIDS would therefore be useful to these patients. It is known from literature that nutrient requirements for HIV-positive/AIDS patients are higher than those of HIV-negative individuals due to the catabolic activity of HIV infection/disease, and in most cases scientists have suggested dietary intakes higher than the RDA for HIV-positive/AIDS persons (Sappey *et al*., 1994; Fawzi *et al*., 1998; Piwoz & Preble, 2000). The median magnesium intake reported for this study population is significantly higher than the RDA. This adequate intake can therefore help in preventing complications associated with inadequacy in HIV-positive/AIDS patients.

Selenium, although needed in trace amounts, is important in respective HIV infection because of its role in the metabolism of the essential anti-oxidant glutathione. Selenium also function synergistically with vitamin E in blocking the rate of lipid peroxidation (Cairns, 2001). In this study, selenium intakes of male patients were higher than the RDA, although 12.5% who had an intake of less than 67% of the RDA. In the female patients, selenium dietary intakes were less than the RDA while 48.2% had intakes of less than 67% of the RDA. Selenium deficient HIV-infected persons have been found to be nearly twenty times more likely to die from HIV-related causes than those with an adequate

intake (Chan & Collins, 1997). The mean selenium intake of women in a study in Bloemfontein was reported to be slightly lower than the RDA and about half of the total population took in less than 67% of the RDA (Hattingh, 2001, unpublished result).
An inadequate selenium intake may be a general problem in the African community, probably resulting from an inadequate intake/deficient in their diets, thus further investigation into the reason or reasons for this inadequate intake of selenium among this community is necessary.

The median intake of the water-soluble vitamins (niacin, riboflavin, B_6, B_{12}, C and folate) was higher than the RDA required for both male and female patients. Other studies have documented an adequate or higher intake of these vitamins (Walsh, 2003, unpublished work; Vorster *et al.*, 1997). Higher intakes of micronutrients such as riboflavin, thiamine and niacin have been associated with higher $CD4^{+}$T-cell counts at baseline (Abrams *et al.*, 1993). The current study did not show any correlation between dietary intake for riboflavin, thiamine, niacine and $CD4^{+}$T-cell count. Tang *et al.* (1993; 1996) observed a slower progression of disease and reduced risk of mortality with an increased intake of riboflavin, thiamine and vitamin C.

Vitamin C has been found to affect immune function in several ways (Watson, 1994). It can stimulate the production of interferons; the proteins that protect cells against viral attack. It has also been shown that vitamin C can stimulate the synthesis of humoral thymus factor and antibodies IgG and IgM classes (Watson, 1994; Flodin, 1988). It has been shown showed that vitamin C was effective in the inactivation of a wide range of pathogenic bacteria including *Staphylococcus aureus*, and *Escherichia coli (Watson, 1994; Chan & Collins, 1997)*. The dietary intake of vitamin C was adequate in the patients examined in the current study. There is accumulating epidemiological evidence that increased intakes of vitamin C may help to reduce the risk of diseases associated with increased oxidative stress (Watson, 1994; Oguntibeju *et al.*, 2003). It is therefore envisaged that the adequate vitamin C intake reported among the patients in this study is beneficial to the patients.

Vitamin B_6 and B_{12} indicate no deficiency in the male patients in this study. The inclusion of animal sources in the diet probably eliminates the possibility of B_6 and B_{12} deficiency. The adequate intakes of vitamin B_6 and B_{12} reported in the studied group may contribute to slower progression of HIV infection tality. B_6 is particularly important for immune function and deficiency can lead to decreased white blood cell response and shrinkage of the critical immune system organ, the thymus (Hoffman, 1998; Bowers, 2002). B_{12} is also central to immune processes because it governs cell division and growth. Deficiency of B_{12} negatively affects red cell maturation (Hoffman, 1998).

Folate (folic acid) is known to play an important role in the prevention of megaloblastic anaemia. The relatively high percentage of folate (37.5% and 33.3% in male and female (table 4.3) patients respectively) having a dietary intake of less than 67% of the RDA is therefore a matter of concern in this group of patients. Although, the greater percentage of patients examined in this study had a dietary intake of the water-soluble vitamins higher than the RDA, it should be noted that even a mild state of deficiency of these vitamins could result in an altered immune function, especially in patients who are not on antiretroviral drugs. As HIV infection progresses, coupled with opportunistic infections and metabolic demand, HIV-infected individuals may be unable to meet their required nutritional needs due to decreased oral intake, decreased nutrient absorption, increased nutrient requirements and changes in metabolism and nutrient transport, which could steadily result in greater inadequacies of these vitamins (Dannhauser *et al*., 1999). The percentage of nutrients with an intake of less than 67% of the RDA reported in some of the patients for the above-mentioned vitamins, may be related to their high intake of maize, a poor source of these nutrients, in addition to other biological factors. Dannhauser *et al*., (1999) alluded to the fact that regular consumption of maize-meal could be the cause of an inadequate intake of these vitamins.

In this study, a significantly higher percentage of the patients had a dietary intake of higher than the RDA for B_6, B_{12} and folate. Although the risk of progression of HIV infection to AIDS was not tested in the current, nonetheless, by inference, the higher intake reported in the patients for this study could be considered beneficial to the patients in terms of the risk of HIV progression to AIDS.

Researchers have provided both animal and human evidence that an adequate vitamins A and E intake and their corresponding blood levels are important for modulating normal immune function (Ross, 1996). The dietary intake of other fat-soluble vitamins is reported and discussed in this study. The dietary intake of vitamin A in this study was higher than the RDA for a greater percentage of the patients, with only 12.5% of male and 14.81% of female having an intake of less than 67% of RDA (table 4.3). Karter *et al.* (1995) reported that 12%-19% of HIV-positive patients at various stages of HIV infection showed vitamin A deficiency/inadequacy and that this inadequacy may be more prevalent in women than in men. The reported 12.5% (male) and 14.81% (female) of vitamin A intake of less than 67% of the RDA in this study is similar to the trend reported by Karter *et al.* (1995). On the other hand, Walsh *et al.* (2003, unpublished result) reported a high intake of vitamin A (higher than the RDA) in HIV-positive patients that is similar to the results of this study. Previous study has shown that there is a relationship between the dietary intake of vitamin A, its blood levels and immune function (Van Staden *et al.*, 1998). The high dietary intake of vitamin A observed in the patients may be related to metabolic demand during the acute phase of HIV infection, or an increased dietary intake, while the low intake could probably be associated with a more rapid progression to AIDS (Rich *et al.*, 2000); the presence of local pathological conditions such oral thrush, and a reduced dietary intake, among other factors (Dworkin *et al.*, 1985; Cuff, 1990; Macallan, 1999).

An inadequate vitamin E intake (table 4.3), found in this study population, occurred in females (14.81%), in males (12.5%) and in the whole group of patients (14.3%). Meanwhile the majority of the patients documented an intake of vitamin E that was higher than the RDA. In this population, the same factors tend to influence a high or low dietary intake for most of the vitamins, vitamin E inclusive. In a study (Hogg *et al.*, 1995) of 100 asymptomatic HIV-sero-positive persons, 26% had an intake of vitamin E that was less than the RDA and 27% had an overt or marginally deficient intake. In another study (Cairns, 2001), 50% of 18 AIDS patients, 58% of 12 ARC patients and 38% of 13 HIV-positive persons had an intake of vitamin E less than the RDA. The 14.8% reported in the present study is lower than that reported by Cairns (2001). Factors such as increased dietary intake, differences in methodologies and design, stages of HIV infection and study population could possibly explain the difference in the percentages (high or low) of vitamin E.

There is limited and conflicting evidence on the influence of vitamin D on immunity and HIV infection. Some research suggests that high intake/levels of vitamin D may have a suppressive effect and that it may stimulate HIV replication (Cairns, 2001). This evidence is based on test tube studies (Cairns, 2001). On the other hand, the active form of vitamin

D has been shown to stimulate macrophages and white blood cells. A study (Haug, 1998) compared 54 HIV-infected people with non-HIV-infected controls. Fifty-four percent of the HIV-positive group were deficient in vitamin D in contrast to the control group, who were not vitamin D deficient. Inadequate intake in this study was reported for vitamin D (table 4.3) in 12.5% of male patients and 29.63% of female patients (intake less than 67% of AI). This value was significantly lower in this study than that of Haug (1998), however, the 29.63% inadequate vitamin D intake found in the female group is similar to that reported by Hattingh, 2001, (unpublished results). Significantly, a higher percentage of the patients had a vitamin D intake higher than the required AI.

The median intakes of vitamin K were high, with both male and female patients exceeding the AI. Inadequate intake was found in 25% of male and 11.11% of female patients (table 4.3). Scientific reports on the intake of vitamin K in HIV-positive/AIDS is very limited. An adequate intake of vitamin K may be vital for HIV-positive patients with bleeding disorder. Previous studies carried out on HIV-positive/AIDS patients in the Free State province of South Africa did not analyse the vitamin K intake. However, it was shown to be adequate or higher than the AI in the HIV-negative population in the Free State province (Van Staden *et al.*, 1998; Dannhauser *et al.*, 1999; Hattingh, 2001, unpublished results).

4.6 Conclusion

The results of this study provide comprehensive information (survey data) on the dietary/nutritional intakes of HIV-positive/AIDS patients, particularly those living in Bloemfontein, and perhaps reflect the dietary intakes of HIV-positive/AIDS patients in the African community. Generally, the dietary intakes were higher than the RDA/AI. The study therefore establishes that the EER (estimated energy requirements), macronutrient and micronutrient intakes of most clinically stable AIDS and HIV-sero-positive patients meet the RDA standards. This high intake may be related to employment status, the provision of food security from families, friends and monthly allowances from the government. The high intake could help in correcting nutritional impairment and retard wasting to a certain extent and, in turn, in maintaining the nutritional level of the nutrients in the blood. On the other hand, the high percentage of patients with an inadequate intake of iodine and selenium is of great concern and calls for further investigation.

The cross-sectional nature of the study, the small number of patients studied, the absence of a control group and the lack of references adapted for HIV-positive/AIDS patients, make it difficult to extrapolate the results. Furthermore, in the present study, there is a lack of information on some important predictors of patients' nutritional status, for instance, blood levels of the corresponding nutrients analysed in the study were not determined and therefore prevent one from making casual references or definite conclusions. However, it is suggested that many serum micronutrients are affected by infection and inflammation (hence their blood levels may not reflect a true picture of the nutritional status of HIV-infected/AIDS patients), therefore, the determination of dietary intake thus plays an important role in the assessment of nutritional status of HIV-positive/AIDS patients. Both macronutrients and micronutrients play an important role in maintaining nutritional status, and probably delay disease progression. Irrespective of the availability of anti-retroviral therapy, an adequate, well balanced diet, providing required foods and consequently adequate nutrients to meet the increased requirements of HIV infection/AIDS, is an important measure in maintaining nutritional health in people living with HIV/AIDS.

4.7 Application

Assessment of HIV-positive/AIDS patients is important at all stages of the disease in order to identify those with inadequate dietary intake who will require counselling. The inferences drawn from this study will in no small measure assist the government and health professionals in designing nutritional support for HIV-infected persons. Results from the study may also provide useful information for health professionals who are involved in studies on the nutritional implications of antiretroviral drugs which the government intend to provide in a couple of months from now.

4.8 Recommendations

To avoid malnutrition in people with HIV/AIDS, nutritional counselling is imperative and is therefore suggested in order to assist people with HIV/AIDS to maintain their body weight and quality of life. The nutritional counselling should :

- Provide information on adequate and well balanced diets, including recipes for preparing meals
- Stress the importance of optimising and maintaining nutritional status

- Stress early treatment of opportunistic infections as infections increase the need for nutrients, impair nutrient absorption and reduced dietary intake
- Discuss alternative therapies
- Provide guidelines for home-based assessment of body weight.

4.9 References

Abrams B, Duncan D & Hertz-Picciotto I (1993). A Prospective Study of Dietary Intake and Acquired Immune Deficiency Syndrome in HIV-1 sero-positive Homosexual Men. **J Acquir Immune Defic Syndr 6**: 949-958.

Allard JP, Aghdassi E, Chau J, Tam C, Kovacs CM, Salit IE & Walmsley SL (1998). Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. **AIDS 12**: 1653-1659.

Babamento G & Kotler DP (1997). Malnutrition in HIV infection. **Gastroenterol Clin North Am 26**: 393-415.

Baum MK, Shor-Posner G, Lu Y (1995). Micronutrients and HIV-1 disease progression. **AIDS 9** (9): 1051-1056.

Baum MK & Shor-Posner G (1998). Micronutrient status in relationship to mortality in HIV-1 disease. **Nutrition Reviews 56**: S135-S139.

Bogden JD, Baker H, Frank O (1990). Micronutrient status and human immunodeficiency virus (HIV) infection. **New York Acad Sci 587**: 189-195.

Bowers JM (2002). Nutrition and Immunity. **ACRIA Update 11** (2): 1-4.

Castetbon K, Kadio A, Bondurand A, Boka Yao A, Barouan C *et al.* (1997). Nutritional status and dietary intakes in human immunodeficiency virus (HIV)-infected outpatients in Abidjan, Cote d'Ivoire, 1995. **European J Clin Nutr 51**: 81-86.

Cairns G (2001). Vitamins, minerals and other supplements: Conference Report: 1-63.

Chan G & Collins J (1997). Antioxidants may still have a role in HIV treatment. **Treatment Issues 11** (9):1-7.

Chan G & Collins J (1997). Antioxidants may still have a role in HIV treatment. **Treatment Issues 11** (9): 1-25.

Chleboski RT, Grosvenor MB, Kruger S, Bulcavage L, Beck K, Beall G (1989). Dietary intake of HIV infection: relative caloric deficiency in patients with AIDS. In: proceedings of the fifth International Conference on AIDS. Montreal, Canada (Abst 467).

Cimoch P (1997). Nutritional health. Prevention and treatment of HIV-associated malnutrition: A case manager's guide. International Association of Physicians in AIDS Care. www.iapac.org/clinmgt/nutrition/caseguide.html (Accessed on 16 Nov., 2002)

Cuff PA (1990). Acuired immunodeficiency syndrome and malnutrition: role of gastrointestinal pathology. **Nutr Clin Practice 5**: 43-53.

Dannhauser A, Van Staden AM, Van der Ryst E, Nel M, Marais N, Erasmus E *et al.* (1999). Nutritional status of HIV-1 sero-positive patients in the Free State Province of South Africa: Anthropometric and dietary profile. **European J Clin Nutr 53**: 165-173.

Department of Health, South Africa (2002). Summary report: National HIV and Syphilis antenatal sero-positive survey in South Africa: 1-10

Drewnowski A & Popkin BM (1997). The nutrition transition. New trends in the global diet. **Nutrition Reviews 55** (2): 31-43.

Dworkin BM, Wormser GP, Rosenthal WS, Heier SK (Braunstein M, Weiss L *et al*. (1985). Gastrointestinal manifestations of AIDS: a review of 22 cases. **Am J Gastroenterol 80**: 774-778.

Dworkin BM, Axelrod F, Pierre N, Woemser G, Weiss, Scharz E (1989). An analysis of nutrient in patients with AIDS, AIDS-related complex (ARC) and asymptomatic HIV positive control. **Gastroenterology 96**: 133.

Dworkin BM, Wormser GP, Axelrod . , ... vardz E & Seaton T (1990). Dietary intake in patients with acquired immunodeficiency syndrome (AIDS), patients with AIDS-related complex and serologically positive human immunodeficiency virus patients: correlations with nutritional status. **J Pop Epi Nutr 14**: 605-609.

Fawzi WW & Hunter DJ (1998). Vitamins in HIV disease progression and vertical transmission. **Epidemiology 9** (4): 457-466.

Fawzi WW, Msamanga GL, Spiegelman D (1998). For the Tanzania Vitamin and HIV Infection Trial Team. Randomized trial of effects of vitamin supplements on pregnancy outcomes and T-cell counts in HIV-1-infected women in Tanzania. **Lancet 351**: 1477-1482.

Flodin NW (1988). Pharmacology of micronutrients. Allan Rev Liss Inc 7: 201-244

Friis H & Michaelsen KF (1998). Micronurients in HIV infection: a review. **European J Clin Nutr 52**: 157-163.

Grunfeld C & Feingold KR (1992). Metabolic disturbances and wasting in AIDS. **N Engl J Med 327** (5): 329-337.

Harriman GR (1989). Vitamin B-12 malabsorption in patients with acquired immunodeficiency syndrome. **Annals of Internal Medicine 149** (9): 2039-2041.

Hattingh Z (2001). The relationship between anthropometry, dietary intake and type 2 diabetes mellitus. M.Tech Thesis, Technikon Free State, Bloemfontein pp1-22.

Haug CJ (1998). Severe deficiency of 1, 25-dihydroxyvitamin D3 in human immunodeficiency virus infection: association with immunological hyperactivity and only minor changes in calcium homeostasis. **J Clin Endocrinology & Metabolism 83** (11): 3832-3838.

Hellerstein MK, Kahn J, Mudie, Vit[illegible]. Current approach to the treatment of human immunodeficiency virus-associated weight loss: pathophysiologic considerations and emerging management strategies. **Semin Oncol 17**: 17-33.

Hoffman R (1998). Nutrients to boost immunity: www.consciouschoice.com (Accessed on 7 August 2004).

Hogg RS, Zadra JN, Chan-Yan C (1995). Analysis of nutritional intake in a cohort of homosexual men. **J Acquir Immune Defic Syndr Hum Retrovirol 9**: 162-167.

Jariwalla RJ (1995). Micronutrient imbalance in HIV infection and AIDS: Relevance to pathogenesis and therapy. **J Nutritional & Enviromental Medicine 5** (3):297-204.

Karter DL, Karter AJ, Yarrish R (1995). Vitamin A deficiency in nonvitamin supplemented patients with AIDS: a cross-sectional study. **J Acquir Immunodefic Synd Hum Retrovirol 8**:199-204.

Kotler DP (1997). Management of nutritional alterations and issues concerning quality of life. **J Acquir Defic Syndr & Hum Retrovirol 16** (S1): S30-S35.

Kotler DP, Tierney AR, Brenner S (1990). Preservation of short-term energy balance in clinically stable patients with AIDS. **Am J Clin Nutr 51**: 7-13.

Kim JH, Spiegelman, Rimm Eric & Gorbach S (2001). The correlates of dietary intake among HIV-positive adults. **Am J Clin Nutr 74** (6): 852-861.

Kupka R & Fawzi W (2002). Zinc nutrition and HIV infection. **Nutrition Reviews 60** (3): 6979.

Macallan DC (1999). Wasting in HIV Infection and AIDS. **J Nutrition 129** (1): 238S-242S.

MacIntyre UE (1998). Dietary intakes of Africans in transition in the North-West Province, PhD thesis, Potchefsroom PU for CHE.

Malvy DJM, Richard MJ, Arnaud [illegible]onship of plasma malondialdehyde, vitamin E and antioxidant micronutrients to human immunodeficiency virus-1 seropositivity. **Clinica Chimica Acta 224**: 89-94.

McCorkindale C, Dybevik K, Coulston AM, Sucher KP (1990). Nutritional status of HIV-infected patients during the early disease stages. **J Am Diet Assoc 90**: 1236-1241.

Mocroft A, Kirk O, Barton SE, Dietrich M, Proenca R (1999). Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. **AIDS 13** (8): 943-950.

Nolan D, Upton R, James I (2000). Longitudinal analysis of bone mineral density (BMD) in HIV-infected patients treated with HAART. 2nd International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV, Toronto (Abst 031).

Oguntibeju OO, van Schalkwyk FE, van den Heever WMJ & Veldman FJ (2003). A review of the role of vitamin C in health. **Mary Slessor J Med 3** (2): 75-81.

Piwoz EG & Preble EA (2000). HIV/AIDS and nutrition: a review of the literature and recommendations for nutritional care and support in sub-Saharan Africa. A Technical Report: 1-8.

Phuapradit W, Chaturachinda K, Taneepanichskul S, Sirvarasry J, Khupulsup K & Lerdvuthisopon N (1996). Serum vitaminA and beta-carotene levels in pregnant women infected with human immunodeficiency virus-1. **Obstet. Gynecol 87**: 564-567.

Rich KC, Fowler MG, Mofenson LM (2000). Maternal and infant factors predicting disease progression in HIV-1-infected infants. **Pediatrics 105**: E8.

Ross AC (1996). The relationship between immunocompetence and vitamin A. In: Sommer A, West KE, eds. Vitamin A deficiency: health, survival and vision. New York: Oxford University Press: 251-268.

Sappey C, Leclercq P, Coudray C, [illegible] M &Favier A (1994). Vitamin, trace element and peroxide status in HIV seropositive patients: asymptomatic patients present a severe β-carotene deficiency. **Clinica Chimica Acta 230**: 35-42.

Scrimshaw NS & SanGiovanni JP (1997). Synergism of nutrition, infection and immunity: An overview. **Am J Clin Nutr 66**: 464S-477S.

Semba RD & Tang AM (1999). Micronutrients and the pathogenesis of human immunodeficiency virus infection. **Br J Nutr 81**: 181-189.

Semba RD (1994). Vitamin A, immunity and infections. **Clin Infect Dis 19**: 489-499.

Semba RD, Shah N, Klein RS, Mayer KH, Schuman P (2001). Human Immunodeficiency Virus Epidemiology Research) Study Group. Highly active antiretroviral therapy associated with improved anaemia among HIV-infected women. **AIDS Patient Care STDS 15** (9): 473-480.

Smith PD, Quinn TC & Strober W (1992). Gastrointestinal Infections in AIDS. **Ann Intern Med 116** (1): 63-77.

Sullivan PS, Hanson DL, Chu SY, Jones JL & Ward JW (1998). Epidemiology of anaemia in human immunodeficiency virus (HIV)-infected persons: results from the multistate adult and adolescent spectrum of HIV disease surveillance project. **Blood 91** (1): 301-308.

Tang AM, Graham NMH, Kirby AJ (1993). Dietary micronutrient and risk of progression to acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus type 1 (HIV-1)-infected homosexual men. **Am J Epidemiol 138**: 1-15.

Tang AM, Graham NMH, Saah AJ (1996). Effects of micronutrient intake on survival in human immunodeficiency virus type 1 infection. **Am J Epidemiol 143**: 1244-1256.

Tindall B & Cooper DA (1991). Primary HIV infection: host responses and intervention strategies. **AIDS 5**: 1-14.

USAID (2001). AIDS epidemic update (internet). Joint United Nations Programme HIV/AIDS. New York: http://www.usaids.org/epidemic_update/report_dec01/index.html (Accessed 14 May 2002).

Van Staden AM, Barnard HC, Nel M, Attwood EM, Dannhauser A, Van der Ryst E *et al.* (1998). Nutritional status of HIV-1 sero-positive patients in the Free State Province of South Africa-laboratory parameters. **Cent African J Med 44** (10): 246-250.

Vorster HH, Oosthizen W, Jerling JC, Veldman FJ & Burger HM (1997). The nutritional status of South Africans. A review of the literature from 1975-1996. Durban. **Health Service Trust 1**: 1-48.

Walsh C, Bester I & Veldman D (2001). Dietary intake of HIV-positive women (25-44 years) in Mangaung. A preliminary research report: 1-20.

Watson RR (1994). Nutrition and AIDS. CRC Oress, Boca Raton, London: 120-130.

WHO (2003). Nutrient requirements for people living with HIV/AIDS. Report of a technical consultation. WHO, Geneva: 1-16

Woods MN, Spiegelman D, Knox TA, Forrester JE, Connors JL, Skinner SC *et al.* (2002). Nutrient intake and body weight in a large cohort that includes women and minorities. **J Am Diet Assoc 102**: 203-211.

Woznicki D & D'Alessandro G (1997). Nutrition against AIDS. **Am Council of Sc & Health 9** (2): 1-4.

Chapter 5

Anthropometric Profiles of HIV-positive/AIDS patients at baseline and end of Nutrient Supplementation

5.1 Abstract

This is the first study in the Free State Province of South Africa to have examined the potential influence of a nutrient supplement on the anthropometric profiles of HIV-positive/AIDS patients and the correlation between anthropometric profile, $CD4^{+}$T-cell count and viral load. Anthropometric profiles were determined according to standard procedures in 35 HIV-positive/AIDS patients at baseline and in 28 patients at final visit. At baseline, of the 35 patients recruited into the study, 32 (94.1%) showed a fat percentage below normal range. Twenty-four of the patients (68.6%) had body mass index (BMI) within normal range, while a greater percentage of the patients had a normal waist-to-hip ratio (WHR). Twenty-eight patients completed the study. Of these, 26 (96.3%) reported a fat percentage of below 18.5%. There was no significant difference ($P>0.05$) between the fat percentage at baseline and end of the experiment in the whole group, however, this differed significantly ($P<0.05$) according to gender, being lower in the male group than in the female group. The results showed that 19 (67.9%) had a BMI within the normal range after nutrient intervention. The body weight did not decline significantly ($P>0.05$). In general, the BMI and lean body mass (LBM) produced a trend towards an improvement. At baseline visit, the viral load showed a significant correlation with the BMI, and the $CD4^{+}$T-cell count significantly correlated with fat percentage. As expected, there was a significant positive correlation between the BMI and fat percentage. At the final visit, the $CD4^{+}$T-cell count showed no correlation with any of the anthropometric indices while the viral load showed a significant negative correlation with the LBM and BMI. The short duration of the study probably limited the positive trend of the supplement.

5.2 Introduction

Infection with the human immunodeficiency virus (HIV) has a devastating effect on the nutritional status of infected persons. Weight loss, often profound in magnitude depending on the stage of the infection and associated infections, are outstanding features of HIV infection. HIV-positive/AIDS patients may lose 30-50% of their body mass before progressing to AIDS (Gorbach & Knox, 1992). According to different researchers, weight loss can be caused by five mechanisms, namely, inadequate food intake, reduced intestinal absorption, abnormal utilization, increased excretion of nutrients and increased host requirements (Herbert, 1973; Kotler *et al.,* 1989; Grunfeld & Feingold, 1992; Macallan, 1999). It is also agreed that weight loss contributes to the progression of HIV infection to AIDS (Chlebowski, 1985; Dannhauser *et al.*, 1999).

Malnutrition has been an endemic problem in Africa for decades and it is complicated by a combination of factors and more recently by the impact of HIV/AIDS (Piwoz & Preble, 2000). HIV/AIDS and malnutrition are interrelated. Research suggests that malnutrition increases the progression of HIV infection and in turn HIV infection exacerbates malnutrition through its attack on the immune system and body composition (Fawzi & Hunter, 1998; Friis & Michaelsen, 1998; Oguntibeju *et al.,* 2003a). The relationship between malnutrition and HIV/AIDS is well recognised. In fact, in Africa, AIDS was initially known as the "Slim Disease" because of the classical wasting condition experienced by persons with HIV disease (Piwoz & Preble, 2000).

To understand the relationship between HIV infection and nutritional status, one must consider the effect of the infection/disease on the body size and composition (weight, lean body mass and body cell mass). In populations in which malnutrition is endemic (as it is in Africa), body size and composition changes are associated with protein-energy malnutrition (Babamento & Kotler, 1997). Weight loss and body composition changes are said typically to follow two patterns in people living with HIV/AIDS. This includes slow and progressive weight loss resulting from anorexia and gastrointestinal disturbances, and rapid, episodic weight loss following secondary infections. It has been observed that even relatively small losses in weight (5%) have been associated with decreased survival in individuals with AIDS (Macallan, 1999).

The earliest studies of nutritional status in AIDS patients conducted between 1981 and 1983 were undertaken with hospitalized patients. Weight loss to an average of about 80% of ideal weight was found. Evidence of protein deficiency was documented by showing deficiencies in serum proteins and muscle wasting (Kotler *et al*., 1989 & 1996). Other studies also documented a very high prevalence of severe weight loss in AIDS patients at the time of admission (Serwadda *et al*., 1985; O'Sullivan *et al*., 1985; Niyongabo *et al*., 1999).

Studies by Kotler *et al.* (1985), mostly with symptomatic HIV-positive patients with advanced disease, found that total body potassium, an index of body cell mass, was reduced in patients who were compared to controls. Body fat was also depleted, but to a lesser extent than body potassium, indicating a greater depletion of lean body mass in the immuno-deficient patients. In more stable ambulatory patients with AIDS, however, the researchers found that weight loss was not associated with reductions in lean body mass, body fat or total water. The researchers noted that the cause of weight loss in these patients was unclear. On the other hand, in a small group of HIV-infected women, loss of body fat was greater than loss of lean body mass (Macallan, 1999).

The aims of nutritional support or supplementation are to provide adequate levels of all nutrients; to preserve lean body mass and functional status; to improve quality of life, and to maximise response to medical therapy (Woznicki & D'Alessandro, 1997; Oguntibeju *et al.,* 2003b). According to Oliver and Hyder (1992), it should be a general practice to supplement all HIV-positive persons at all clinical stages, especially those with a low socio-economic status. Appropriate and timely nutritional intervention may reverse the weight loss caused by problems with food ingestion and may consequently result in an increase in weight and body cell mass (Kolter *et al.,* 1990; 1991; Summerbell, 1994). Available studies suggest that nutritional supplementation is effective in restoring the lean body mass of HIV-infected persons especially in the absence of any chronic infection (Calderon *et al*., 1990; Gorbach & Knox, 1992).

Research has shown that in the early period of HIV infection, weight gain or maintenance might be achieved through nutrition intervention, and this has helped to reduce the consequences of wasting in people living with HIV/AIDS (Friis & Michaelsen, 1998; Dwyer, 1998). In the developing world, where the majority of people living with HIV cannot afford antiretroviral therapy, good nutrition combined with mineral and vitamin supplementation could be a good source of therapy. Malnutrition is known to favour opportunistic infections and contributes to wasting (Cimoch, 1997; Niyongabo *et al.*, 1997), making supplementation an important aspect in the management of people living with HIV/AIDS. Nutritional intervention may help to strengthen the immune system and reduce the severity and impact of opportunistic infections in people living with HIV/AIDS (Raiten, 1990; Woznicki & D'Alessandro, 1997; Dwyer, 1998).

Some studies (Dannhauser *et al.*, 1999; Niyoganbo *et al.*, 1999) have examined the relationship between HIV infection and adult anthropometry in South Africa and elsewhere in Africa. However, none has examined the association between HIV infection, anthropometry and nutrient supplementation in adult male and female populations. This paper examined the influence of nutrient supplementation on the anthropometric profile of HIV-positive/AIDS patients in Bloemfontein, South Africa, so as to establish whether nutrient supplementation has any influence on the anthropometric indices of HIV-positive/AIDS patients.

5.3 Methodology

The study consisted of a baseline visit and a monthly visit that lasted from April to September 2003. The duration of this study is similar to that of Allard *et al.* (1998). An approval from the Ethics Committee of the Faculty of Health Sciences, University of the Free State was obtained (ETOVS 32/03). The patients signed the consent form at their first visit after they had been told the purpose and protocol of the study. The inclusion criteria included male and female patients between 18 and 65 years of age that were HIV positive; willing to undergo a pre-study physical examination; were not on antiretroviral therapy; had been found to be within the range of clinical acceptability in medical history and physical examination; had a $CD4^{+}$T-cell count of 100-350 cells/mm^3, and were able to comprehend; and willing to sign, the statement of informed consent.

5.3.1 Sample Collection

This is described in chapter 3 under methodology (see **3.5.5.1**)

5.3.2 Anthropometric measurements

At baseline and by the end of the study, the following anthropometric indices were determined and included body mass index (BMI) calculated from body weight and height in metres squared; waist-hip-ratio (WHR) calculated from waist circumference, and hip circumference and skin-fold thickness (to estimate percentage of body fat). For the anthropometric measurements, the subjects presented themselves in minimal clothing to allow measurements to be done quickly, accurately and efficiently. Two persons were involved with the measurement: the measurer and the recorder. This was done to ensure accuracy of site location, correct sequence of measurement sites and accurate reading. The recorder repeats the value as it is being recorded in order to enable the measurer to do an immediate check. The measurement was done twice at each site on each subject and the average value was taken. Throughout the marking and measurement session, each subject stood relaxed, with arms comfortably by the side and with feet together. However, a few measurements required the subjects to place their feet apart. During the measuring period, the measurer was able to move around the subject easily and also to manipulate the equipment. All anthropometric measurements were done according to standard procedures (Lee & Nieman, 1993; 1996; Laquatra, 2000).

5.3.3 Supplementation

This is described in chapter 3 under methodology (see **3.4.2**).

5.3.4 Follow-up Visits

This is described in chapter 3 under methodology (see **3.5.4.2**)

5.4 Results

The results of the baseline visit of 35 patients are indicated in Table 5.1. Thirty-two of the respondents (94.1%) had a fat percentage below 20% while 2 (5.9%) had fat percentage above 25%. One patient (female) was excluded from fat percentage due to cut-off range for age.

Of these 35 patients, 8 (22.9%) had an 18.5 kg/m^2, 24 (68.6%) had a BMI range of 18.5-24.9, while 3 (8.6%) of the respondents had a BMI greater than 25. Seven (87.5%) of the respondents reported a WHR of less than 0.95 and 1 (12.5%) of the respondents reported a WHR of greater than or equal to 0.95 for males. In the female group, 13 (48.1%) of the respondents reported a WHR of less than 0.8 while 14 (51.9%) reported a WHR of greater than or equal to 0.8 (table 5.1).

The results of the final visit of 28 patients are indicated in Table 5.1. The result showed that 26 (96.3%) of the patients reported fat percentage of below 20 while one had fat percentage of above 25%. One patient was excluded from fat percentage due to cut-off range for age.

Of the 28, 7 (25%) had a BMI of less than 18.5 kg/m^2, 19 (67.9%) had a BMI within the range of 18.5 kg/m^2-24.9 kg/m^2, and 2 (7.1%) had a BMI greater than or equal to 25 kg/m^2. Seven (87.5%) of the patients had a WHR of less than 0.95 and 1 (12.5%) had a WHR of greater than or equal to 0.95. Ten (50%) had a WHR of less than 0.8 and 10 (50%) had a WHR greater than or equal to 0.8 for female patients (table 5.1).

Table 5.1: The frequency and percentage of body fat, BMI and WHR of HIV-positive patients at baseline (n=35) and at final visit (n=28).

Anthropometric profile	Baseline visit n=35	Final visit n=28
	Freq & percentage	**Freq & percentage**
Fat %*		
<20	32 (94.1%)*	26 (96.3%)*
20≤25	0 (0%)	0 (0%)
>25	2 (5.9%)	1 (3.7%)
BMI (kg/m^2)		
<18.5	8 (22.9%)	7 (25%)
18.5-24.9	24 (68.6%)	19 (67.9%)
≥25	3 (8.6%)	2 (7.1%)
WHR		
<0.95 (male)	7 (87.5%)	7 (87.5%)
≥0.95 (male)	1 (12.5%)	1 (12.5%)
<0.8 (female)	13 (48.1%)	10 (50%)
≥0.8 (female)	14 (51.9%)	10 (50%)

*One patient (female) was excluded from fat percentage due to cut-off range for age.

Table 5.2: Anthropometric profiles of HIV-positive/AIDS patients at baseline visit and final visit.

Anthropometric indices	Before nutrient supplementation (n=35)					After nutrient supplementation (n=28)				
	Mean	SD	Median	25 percentile	75 percentile	Mean	SD	Median	25 percentile	75 percentile
Weight (kg)	55.2	8.2	57.0	49.0	60.0	55.7	7.4	56.0	50	60.0
Height (m)	1.6	0.1	1.6	1.55	1.7	1.6	0.1	1.6	1.6	1.7
Forearm (cm)	22.2	2.3	22.0	21.0	24.0	22.7	2.0	22.0	21.5	1.7
Calf (cm)	31.6	4.1	31.5	28.5	35.0	31.4	3.7	32.0	28.0	34.8
Thigh (cm)	39.9	7.2	40.0	35.0	46.0	40.9	7.1	40.5	35.8	45.0
Waist (cm)	73.9	5.7	73.0	70.0	76.0	73.9	5.9	74.0	70.0	76.0
Hip (cm)	91.9	8.4	91.0	85.0	99.0	91.0	7.9	91.3	86.0	96.5
Triceps (cm)	11.5	3.5	11.0	9.5	13.5	11.3	3.4	11.0	9.5	12.0
Subscapular (cm)	10.7	2.8	10.5	9.0	12.5	10.2	2.8	10.0	8.3	11.3
Iliac crest (cm)	10.9	4.2	10.5	8.0	13.0	10.7	3.9	10.0	9.0	12.3
Abdominal (cm)	11.9	3.5	11.0	10.0	14.5	11.8	3.6	10.8	10.0	13.8
Chest (cm)	6.3	1.9	6.0	5.6	7.0	6.4	2.1	6.0	5.3	7.0
Front thigh (cm)	14.6	4.2	14.0	11.5	16.5	14.8	4.6	13.5	11.5	17.0
Medial calf (cm)	11.2	4.1	11.5	7.0	14.0	10.9	4.2	10.0	7.5	14.3
Mid-axilla (cm)	9.9	3.7	9.5	7.0	12.0	9.9	3.4	10.0	7.5	11.3
% fat	17.8	4.7	19.2	13.6	20.3	17.2	4.9	17.9	12.6	20.7
BMI (kg/M^2)	21.2	3.3	20.4	18.7	23.2	21.2	3.4	20.7	18.8	23.2
WHR	0.8	0.1	0.8	0.8	0.8	0.8	0.1	0.81	0.8	0.9
LBM (kg)	45.2	6.2	45.5	40.4	49.3	45.7	5.4	46.1	41.3	49.6

The mean, median, standard d[illegible]centile and 75 percentile of the anthropometric indices are presented in Table 5.2. The median fat percentage of 19.2% at baseline was not significantly lower than the normal range (20≤25%). The fat percentage had not decreased significantly (P>0.05) by the end of nutrient supplementation (table 5.2). The median BMI of the population (inclusive of male and female adults) fell within the accepted/normal range of 20 kg/m^2 and less than 25 kg/m^2 (Laquatra, 2004). At baseline, the median BMI was 20.4 kg/m^2 (within a normal range). It showed a slight trend towards an insignificant increase to 20.7 kg/m^2 (P>0.05) following supplementation. The median WHR of 0.81 showed normal waist circumference of the study population without gender differentiation. The median WHR was maintained up to the end of nutrient supplementation (table 5.2). The median LBM increased insignificantly (P>0.05) from 45.5 kg at baseline to 46.1 kg by the end of nutrient supplementation (table 5.2).

Table 5.3A: Anthropometric variables between gender at baseline visit.

Indices	Mean	SD	Median	25 percentile	75 percentile
Male n=8					
% fat	11.0	1.6	11.0	10.0	12.0
WHR	0.8	0.1	0.8	0.8	0.9
Female n=27					
% fat	19.9	2.9	20.1	17.9	21.4
WHR	0.8	0.1	0.8	0.8	0.8

Table 5.3B: Anthropometric variables between gender after nutrient supplementation (final visit).

Indices	Mean	SD	Median	25 percentile	75 percentile
Male n=8					
% fat	10.9	1.7	10.9	9.7	12.4
WHR	0.8	0.1	0.8	0.8	0.8
Female n=27					
% fat	19.9	2.8	19.7	17.6	21.1
WHR	0.8	0.1	0.8	0.8	0.8

Table 5.3A displays anthropometric variables between genders at baseline. A median fat percentage of 11.0% was reported for male patients. This value is significantly ($P<0.05$) less than 20% and significantly ($P<0.05$) lower than the value reported for the female patients. On the other hand, the fat percentage of 20.1% for the female patients falls within the normal range of 20 less than or equal to 25 (Laquatra, 2004). This value probably reflects a wasting process (sub-clinical in the female) but shows a slight declining trend in the male patients. For the male and female patients, there was no significant difference ($P>0.05$) in the median WHR. Values less than 0.88 indicated normal female waist circumference. Therefore, it could be said that the female patients reported normal waist circumference indicating peripheral fat distribution. The median WHR of 0.8 (table 5.3A) as reported in male patients is lower than normal range of <0.95.

The median fat percentages of the patients were relatively maintained at the end of nutrient supplementation (Table 5.3B). The WHR showed no significant difference ($P>0.05$) between gender at both baseline and end of study. The nutrient supplementation may have possibly reduced further wasting in these patients.

The correlation between anthropometric indices, CD4$^+$T-cell count and the viral load at baseline and by the end of nutrient supplementation were determined and are presented below (table 5.4)

Table 5.4: Correlation between different anthropometric profiles, CD4$^+$T-cell count and viral load at baseline and final visit

Correlation Between		Baseline visit n=35		Final visit n=28	
Variable 1	**Variable 2**	**r**	**p-value**	**r**	**p-value**
LBM	% fat	0.036	0.84	-0.08	0.7
	BMI	0.39	0.02*	0.14	0.5
	WHR	-0.13	0.45	-0.07	0.7
	CD4$^+$T-cell count	0.25	0.2	0.08	0.6
	Viral load	-0.37	0.04*	-0.45	0.02*
% fat	BMI	0.74	<0.0001***	0.81	<0.0001***
	WHR	-0.38	0.02*	-0.4	0.05*
	CD4$^+$T-cell count	0.06	0.74	0.006	0.97
	Viral load	-0.39	0.03*	-0.25	0.2
BMI	WHR	-0.45	0.006*	-0.31	0.1
	CD4$^+$T-cell count	0.2	0.32	0.02	0.9
	Viral load	-0.61	0.0002*	-0.5	0.008*
WHR	CD4$^+$T-cell count	-0.07	0.7	0.05	0.7
	Viral load	0.4	0.01**	0.3	0.2
CD4$^+$T-cell count	Viral load	-0.3	0.2	-0.21	0.3
Viral load	CD4$^+$T-cell count	-0.3	0.21	-0.21	0.3

NS (not significant): P>0.05

*: P<0.05

**: P<0.01

***: P<0.001.

Baseline visit

This study did not indicate a significant correlation between the LBM and percentage of body fat (P>0.05), the WHR (P>0.05) and the CD4$^+$T-cell count (P>0.05) (table 5.4). However, the results showed a positive significant correlation between the LBM and the BMI (P<0.05) and the viral load (P<0.05). The fat percentage showed a positive and a significant correlation with the BMI (P<0.0001) but a negative significant correlation with the WHR (P<0.05) and viral load (P<0.05). There was no significant correlation between the fat percentage and the $CD4^{+}$T-cell count (P>0.05). The BMI indicated a negative correlation with the WHR (P<0.05) and viral load (P<0.0002). There was no significant correlation observed between the BMI and the $CD4^{+}$T-cell count (P>0.05). There was a positive significant correlation between the WHR and the viral load (P<0.1) but not so with the $CD4^{+}$T-cell count (P>0.05). The $CD4^{+}$T-cell count did not correlate significantly with the viral load (P>0.05) (table 5.4).

When the result was analysed according to gender, in the male group; the $CD4^{+}$T-cell count significantly correlated with the fat percentage (r=0.77, P<0.05) and showed a positive association with the BMI.

In the female group, a different pattern was observed. The LBM correlated positively with the fat percentage (r=0.57, P<0.002), and the BMI (r=0.89, P<0.0001) while the fat percentage showed a positive and a significant correlation with the BMI (r=0.59, P<0.001) The LBM (r= -0.64, P<0.001), fat percentage (r= -0.53, P<0.05) and the BMI (r= -0.69, P<0.0002) showed a negative correlation with the viral load while the WHR (r=0.055, P<0.05) showed a positive correlation with the viral load. There was no significant correlation between the $CD4^{+}$T-cell count and the anthropometric profiles or between the $CD4^{+}$T-cell count and the viral load.

Final visit:

In the final visit, there was no significant correlation between the LBM and the fat percentage, the BMI, the WHR, the $CD4^{+}$T-cell count and the viral load (table 5.4). The fat percentage correlated significantly (positively) with the BMI (P<0.0001) and correlated negatively with the WHR (P<0.05). The BMI showed a significant negative correlation with the viral load (P<0.05). There was no significant correlation between the $CD4^{+}$T-cell count and the viral load.

In the male group, the BMI indicated a positive association with the fat percentage (r=0.64) (data not shown in a table). Other anthropometric indices showed no correlation. There was no correlation between the $CD4^{+}$T-cell count, the viral load and the anthropometric indices. In the female group, the LBM showed a positive association with fat percentage (r=0.51, P<0.05), the BMI (r=0.82, <0.0001) and a significant negative correlation with the viral load (r= -0.75, P<0.05) (data not shown in a table). The fat percentage correlated significantly (positively) with the BMI (r=0.59, P<0.05). The $CD4^{+}$T-cell count showed no significant correlation with any of the anthropometric indices while the viral load showed a significant (negative) correlation with the BMI (r= -0.79, P<0.0001).

5.5 Discussion

Studies in developed countries have reported the impact of HIV infection on nutritional status at different stages of the infection (Kotler *et al.*, 1989; Ott *et al.*, 1993). It is reported that malnutrition is a general problem among HIV-infected patients, but it has become much less frequent among HIV-infected persons in developed countries, mainly due to the provision of highly active antiretroviral therapy (HAART) and nutritional interventions (Carbonnel *et al.*, 1998). There have been increasing recommendations for supplementation to form part of the general nutritional interventions to correct some of the nutritional complications associated with HIV infection. The goals of nutritional assessment and intervention are aimed at improving nutritional status, prolonging survival and enhancing quality of life.

In this study, anthropometric indicators of HIV-positive/AIDS patients were determined at baseline and the end of the nutritional supplementation so as to be able to observe the potential influence of the nutritional supplement on the anthropometric indicators. The body weight of the patients had not declined significantly by the end of nutrient supplementation. This finding agrees with the trend reported by Parisien *et al.* (1993) for the body weight of HIV-positive/AIDS patients. Studies have shown that weight loss and decrease in body weight have been indicated as signs of a deterioration of nutritional status in HIV-positive/AIDS patients (Myers, 1997; Dannhauser *et al.*, 1999; Macallan, 1999). In a study, McCorkindale (1990) reported that seven patients at an advanced stage of HIV infection did not show a significant decline in body weight or fat percentage. His patients were on an antiretroviral drug (AZT) for five months. This trend of change underscores the different but yet unclear mechanisms involved in the wasting process in HIV infection. However, in his study although the patients were not on any antiretroviral therapy, the supplement did not demonstrate an observable effect on the body weight. Other factors may explain why the expected weight gain did not occur. Firstly, patients with HIV infection/AIDS may be hypermetabolic, therefore the anticipated weight gain is an overestimation because energy needs and expected weight gain are calculated on the basis of normal metabolic states (Melchoir *et al.*, 1991). Secondly, HIV infection disrupts the normal lipid and protein metabolism which causes nutrients to be used inefficiently and wasted. For example, increased lipogenesis by the liver increases the thermogenesis of food (Hellerstein *et al.*, 1993).

According to Wiley & Samuel (1989); Serwadda *et al.* (1985) and O'Sullivan *et al.* (1985), weight loss and/or a decrease in body weight is a prominent feature of HIV infection/AIDS. O'Sullivan *et al.* (1985) noted that of the 50 HIV-positive/AIDS patients admitted to hospital, despite the fact that their pre-illness weights were higher than normal body weights, 59% of the patients were classified as moderately depleted and 62% had lost >10 of their pre-illness weight. In this study, the pre-illness weights of the patients were not determined because patients were unable to recall their pre-illness weights. The reduction in weight of the patients by the end of nutrient supplementation was not significant. If Serwadda's *et al.* (1985) report did reflect the process of nutritional depletion taking place in HIV-positive/AIDS patients as reflected in the reduction of weight, then we are tempted to believe that the supplement probably has a positive but not visible effect on the weight of the patients. In other words, since the patients were not on antiretroviral therapy, weight reduction or reduction in other anthropometric indicators should have been higher than observed. Likewise, nutritional deterioration of the patients should have been clearly demonstrated in the clinical conditions of the patients. The clinical conditions improved during the course of nutrient supplementation (data on clinical conditions is presented in chapter 6).

The percentage of body fat determined anthropometrically at baseline had not changed significantly by the end of the study (table 5.1). The change in percentage of body fat was significant according to gender, being higher in the females than in the males, indicating that the male patients tended to be leaner than the female patients in this study. This result is similar to that reported by Dannhauser *et al.* (1999). At baseline 94.1% of the population reported fat percentage below the 18.5%, while 96.3% of the population (table 5.1) reported fat percentage by the end of the study, thus the fat percentage showed no significant depletion. This pattern of depletion resembles a stressed or injured state rather than starvation or semi-starvation that is associated with nitrogen saving and increased fat utilization. The result showed a positive correlation between the BMI and fat percentage while the latter did not show a significant correlation with the CD4⁺T-cell count, but a negative correlation with the viral load. Generally, the BMI, LBM and WHR were maintained over the course of the study (table 5.2).

The fact that in this population of HIV-positive/AIDS patients, the LBM was relatively preserved possibly suggests that no major aggressive factor was obviously present. One previous study (MacClave *et al.*, 1992) identified an aggressive factor (Tuberculosis-TB) that significantly contributed to the severity of malnutrition. TB was present in 6 of our patients (17.1%) at baseline; it reduced to 5 (16.7%) at visit one, 2 (6.9%) at visit two and none at visit three, but the patients with TB were treated during the course of the study. It is presumed that the treatment reduced the aggressive impact of TB on the lean body mass. The results seem to show that the wasting process was curtailed, probably to a certain degree in the presence of the supplement and other factors such as the TB treatment. It is believed that the wasting process would have been more aggressive in the absence of the study supplement and treatment because during active phases of infection, people with HIV/AIDS lose LBM rapidly (Gorbach & Knox, 1992).

The probability of imminent death is high when the lean body mass falls to 54% of normal value (Kotler *et al.*, 1996). Clinical conditions associated with wasting include disseminated cytomegalovirus infection, cryptosporidiosis, microsporidiosis, toxoplamosis, kaposis sarcoma, lymphoma and dementia. Most of these conditions were not present in our patients. It is therefore beneficial to study and understand the underlying pathophysiological mechanisms involved in the wasting process which negatively affect the anthropometric indicators of HIV-positive /AIDS patients. The results obtained in this study for the BMI and LBM are similar to those reported by Kennedy *et al.* (2001) following nutrient supplementation. The BMI significantly correlated with the LBM. The LBM showed no correlation with the $CD4^{+}$T-cell count. The viral load significantly correlated with the LBM.

The BMI and fat percentage were lower in patients with $CD4^{+}$T-cell counts <200cells mm/^{3} than in those with $CD4^{+}$T-cell counts >200 cells mm/^{3}. This is understood and expected in patients with immunodeficient diseases such as AIDS, especially when the patients are not on antiretroviral therapy. This finding is similar to that reported by Castetbon *et al.* (1997) and Dannhauser *et al.* (1999). It has been shown that a decrease in the lean body mass is related to the decrease in body cell [illegible] *et al.*, 1997) and that body cell mass depletion is out of proportion to losses of body weight for fat (Kotler, 1992; Kotler *et al.*, 1996; Kotler, 1997). It has also been shown that death from wasting in AIDS is related to the body cell mass depletion rather than to the specific underlying cause of the wasting (Kotler *et al.*, 1989). As previously demonstrated by anthropometry, decline in weight and BMI have been associated with a decline in percentage of body fat.

Although a greater percentage of patients (68.6%) had a BMI within a range of 18.5-24.9, 22.9% of the patients had a BMI of less than 18.5 (table 5.1), therefore for those patients with a lower BMI, reduced body fat may have contributed. It is expected that as the HIV infection/disease progresses, wasting may become more pronounced, which in most cases is reflected in the amount of fat loss or lean body mass. In this study, instead of the wasting continuing significantly, as would have been indicated by a significant decline in the anthropometric indicators, it was relatively curtailed. It is possible that certain factors such as malabsorption, non-compliance, short duration, and drug-nutrient interactions contributed to the non-significant effect of the supplement on the anthropometric indicators (Pronsky *et al.*, 2001).

In a population in which antiretroviral therapy is not readily available or accessible, the findings that nutrient supplementation more or less maintained the BMI or LBM or has the potential of maintaining the BMI or LBM, lends hope for a relatively inexpensive treatment to improve the quality of life and the general well-being of HIV-positive patients.

5.6 Conclusion

The body weight did not decline significantly. In general, the BMI, WHR and LBM produced a trend towards an improvement. The short duration of the study and small sample size probably limited the positive trend of the supplement.

5.7 Limitation of the study

It is possible that certain factors such as malabsorption, short duration and drug-nutrient interaction (for example, TB treatment) could have limited the potential positive effect of the supplement on the anthropometric indicators. In addition, the small sample size and lack of a control group limited the interpretation of the results of this study.

5.8 Application

Nutritional abnormalities are frequent and a characteristic feature of infection with the human immunodeficiency virus (HIV), and they represent a major determinant of survival. Nutritional status may have an impact at all stages of HIV infection: from the initial acquisition of infection, clinical manifestations, progression of the disease and palliation of advanced disease. Nutritional care of HIV-infected patients should therefore begin at the time of diagnosis so as to assess baseline nutritional status and to provide dietary counselling/guidelines. Dietary guidelines should include the recommendation of a nutrient supplement containing anti-inflammatory agents and antioxidants. Close monitoring of nutritional status and clinical status is essential in identifying nutritional problems.

5.9 Recommendations

The following recommendations are made in the light of the results obtained from this study:

- Nutritional intervention should begin early in patients with HIV infection and AIDS.
- Nutritional supplementation should form an important aspect of the clinical management of HIV-positive/AIDS patients as it may help to strengthen the immune system, replace lost vitamins/minerals and reduce the severity of impact of opportunistic infections in people living with HIV/AIDS.
- Standardised evaluation of nutritional status should be done regularly.
- In future, supplementation-based research studies should be directed towards patients with HIV infection and AIDS, with secondary infections and with and without wasting.
- Confirmation of the results of our study is required using the same dietary and patient-entry criteria, and a control group but for a longer period, for example one year.

5.10 References

Allard JP, Aghdassi E, Chau J, Kovacs CM, Salit IE (1998). Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. **AIDS 12**: 1653-1659.

Babamento G & Kotler DP (1997). Malnutrition in HIV infection. **Gastroenterol Clin North Am 26**: 393-415.

Carbonnel F, Maslo C, Beaugerie L, Carat F, Wirbel E, Aussel C *et al.* (1998). Effect of indinavir on HIV-related wasting. **AIDS 12**: 1777-1784.

Calderon E, Ramirez MA, Arrieta MI (1990). Nutrition disorders in HIV disease. **Science 14**: 371-402

Castetbon K, Kadio A, Bondurand A, Boka Yao A & Barouan C (1997). Nutritional status and dietary intakes in human immunodeficiency virus (HIV)-infected outpatients in Abidjan, Cote d'Ivoire, 1995. **European J Clin Nutr 51**: 81-86.

Chlebowski RT (1985). Significance of altered nutritional status in acquired immune deficiency syndrome. **Nutr Cancer 7**: 85-91.

Cimoch P (1997). Nutritional health. Prevention and treatment of HIV-associated malnutrition: A case manager's guide. International Association of Physicians in AIDS Care. www.iapac.org/clinmgt/nutrition/caseguide.html (Accessed on 19 September 2003)

Dannhauser A, Van Staden AM, Van der Ryst E, Nel M, Marais N, Erasmus E *et al.* (1999). Nutritional status of HIV-1 sero-positive patients in the Free State Province of South Africa: Anthropometric and dietary profile. **European J Clin Nutr 53**: 165-173.

Dwyer D (1998). Unproven Nutrition Therapies for AIDS: What is the Evidence? **Nutrition Today 13**: 25-33.

Fawzi WW & Hunter DJ (1998). disease progression and vertical transmission. **Epi 9**: 457-466.

Friis H & Michaelsen KF (1998). Micronutrients in HIV infection: a review. **European J Clin Nutr 52**: 157-163.

Gorbach S & Knox T (1992). Weight loss and human immunodeficiency virus infection: cachexia versus malnutrition. **Infect Dis Clin Pract 1**: 224-229.

Grunfeld C & Feingold K (1992). Metabolic disturbances and wasting in the acquired immunodeficiency syndrome. Symposium: nutritional immunomodulation and AIDS. **J Nutr 122**: 723-727.

Hellerstein MK, Grunfeld C, Wu K, Christiansen M, Kaempfers S, Kletke C *et al.* (1993). Increased de novo hepatic lipogenesis in human immunodeficiency virus infection. **J Clin Endocrinol Metab 76**:559-565.

Herbert V (1973). The five possible causes of all nutrient deficiency: illustrated by deficiencies of vitamin B12 and folic acid. **Am J Clin Nutr 26**: 77-86.

Kennedy OC, Coutsoudis A, Kuhn L (2001). Effects of vitamin A supplementation during pregnancy and early lactation on body weight of South African HIV-infected women. **J Health Popl & Nutr 19** (3): 167-176.

Kotler DP (1992). Nutritional effects and support in the patient with acquired immunodeficiency syndrome. **J Nutr 122**: 723-727.

Kotler DP (1997). Management of nutritional alterations and issues concerning quality of life. **J Acquir Defic Syndr & Hum Retrovirol 16** (1): S30-S35.

Kotler DP, Engelson ES, Thea DM, Wang J, Pierson RN, Saint-Louis M *et al.* (1996). Different body composition changes in men and women with AIDS> X1 International Conference on AIDS. 8-11 July, Vancouver, Canada. Abstract no. MO.B.421.

Kotler DP, Tierney AR, Wang J & 89). Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS. **Am J Clin Nutr 50**:444-447.

Kotler KP, Wang J & Pierson (1985). Body composition studies in patients with acquired immunodeficiency syndrome. **Am J Clin Nutr 42**: 1255-1265.

Kotler DP, Tierney AR, Brenner SK, Couture S, Wang J & Pierson RN (1990). Preservation of short-term energy balance in clinically stable patients with AIDS. **Am J Clin Nutr 51**: 7-13.

Kotler DP, Tierney AR, Ferrano R, Cuff P, Wang J, Pierson RN *et al.* (1991). Enteral alimentation and repletion of body cell mass in malnourished patients with acquired immunodeficiency syndrome. **Am J Clin Nutr 53**: 149-154.

Laquatra I (2004). Nutrition for weight management. In: Mahan LK, & Escott-Stump S, (ed). Krause's Food Nutrition & Diet Therapy. 11th Edition, Philadelphia: WB. Saunders Co: 488-493

Lee RD & Nieman DC (1993). Anthropometry. In: Nutrition Assessment, ed. RD Lee & DC Nieman. Oxford: WCB Brown & Benchmark 121-165.

Lee RD & Nieman DC (1996). Nutritional Assessment. 2nd Ed. McGraw Hill comp. 119-126.

Macallan DC (1999). Dietary intake and weight loss patterns in HIV infection. In: Miller TI & Gorbarch SL, eds. Nutritional aspects of HIV infection. New York: Oxford University Press pp 23-34.

MacClave S, Mittoraj MD, Thielmeier KA & Greenburg RA (1992). Differentiating subtypes (hypoalbuminemic vs marasmic) of protein calorie malnutrition: incidence and clinical significance in a University setting. **J Pop Epi Nutr 16**: 337-342.

McCorkindale C, Dybevik K, Coulstom AM & Sucher KP (1990). Nutritional status of HIV-infected patients during early disease stages. **J Am Diet Assoc 90**: 1236-1241.

Melchoir JC, Salmon D, Rigaud D ret E, Detruchis P (1991). Resting energy expenditure is increased in stable malnourished HIV-infected patients. **Am J Clin Nutr 53**: 437-441.

Myers C (1997). Vitamin/mineral supplementation & HIV/AIDS. **Nutrition Series 7**: 1-3.

Niyongabo T, Henzel D, Ndayishimyie JM, Melchoir JC, Ndayiragije A *et al.* (1999). Nutritional status of adult inpatients in Bujumbura, Burundi (impact of HIV infection). **European J Nutr 53**: 579-582.

Niyongabo T, Bouchaud O, Henzel D, Melchior JC, Samb B Daza, MC *et al.* (1997). Nutritional status of HIV-1 sero-positive subjects in an AIDS clinic in Paris. **Eur J Clin Nutr 51**: 637-640.

O'Sullivan P, Linke RA, Dalton S (1985). Evalaution of body weight and nutritional status among AIDS patients. **J Am Diet Assoc 85**:1483-1484.

Oguntibeju OO, Veldman D & Van Schalkwyk F (2003a). Nutritional status of people living with HIV/AIDS: The situation in Africa: A Review. **J Biomed & Lab Sci 15** (3): 63-67.

Oguntibeju, OO, Van Schalkwyk FE, van den Heever WMJ & Veldman FJ (2003b). The importance of vitamin/mineral supplementation in HIV/AIDS patients to improve their nutritional and immunological status: Short Communication. **Pak J Med Sci 19** (3): 217-219.

Oliver C & Hyder T (1992). Nutrition in human immunodeficiency virus (HIV) disease. Australian **J Nut Diet 49**: (1): Suppl.

Ott M, Lembeke B, Fischer H, Jager R, Polat H, Geier H (1993). Early changes of body composition in human immunodeficiency virus-infected patients: tetrapolar body impedance analysis indicates significant malnutrition. **Am J Clin Nutr 57**: 15-19.

Parisien C, Gelinas MD & Cossette M (1993). Comparison of anthropometric measures of men with HIV: asymptomatic, symptomatic and AIDS. **J Am Diet Assoc 93**: 1404-1408.

Piwoz EG & Preble EA (2000). I [illegible] trition: a review of the literature and recommendations for nutritional care and support in sub-Saharan Africa. A Report 1-4.

Pronsky ZM, Meyer SA, Fields-Gardner C (2001). HIV Medications and Food Interactions. Second Edition. Food-Medication Interactions Publisher: Birchrunville, USA pp 22-26

Raiten DJ (1990). Nutrition and HIV infection: a review and evaluation of the exact knowledge on the relationship between significant malnutrition and HIV infection. **Nutr Clin Pract 6**: 1S-94S.

Serwadda D, Mugerwa RD, Sewankambo NK, Lwegaba A, Carswell JW, Kirya GB *et al* (1985). Slim disease: a new disease in Uganda and its association with HTLV-111 infection. **Lancet 2**: 849-852.

Summerbell C (1994). Appetite and nutrition in relation in HIV infection and AIDS. **Proc Nutr Soc 53**: 139-150.

Wiley JA, Samuel MC (1989). Prevalence of HIV infection in the USA. **AIDS 3** (Suppl 1): S71-78.

Woznicki D & D'Alessandro G (1997). Nutrition against AIDS. **Am Council on Science & Health 9** (2): 1-4.

Chapter 6

The influence of a nutritional supplement on the immune status, haematological parameters and clinical conditions in HIV-positive/AIDS patients

6.1 Abstract

The influence of a nutritional supplement on the immune status, haematological parameters and clinical conditions of HIV-positive/AIDS patients was tested using standard procedures. This clinical trial comprising 35 patients consisted of a baseline visit and three months of supplementation from April to September 2003. The results showed that the viral load decreased significantly ($P<0.002$) with time following supplementation. The mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) increased significantly ($P<0.002$, $P<0.0002$) respectively, reflecting the positive effect of the supplement on a few of the haematological parameters. The supplement showed no effect on the $CD4^{+}$T-cell counts and the $CD4^{+}$T-cell count decreased significantly with HIV disease progression. The supplement also showed observable positive effects on a few of the clinical conditions. Because of certain limitations (small sample size, short duration of study and the final stage of HIV infection of the patients), further studies are needed to confirm the effect of the supplement.

6.2 Introduction

In recent years, the importance of nutrition in human health has received growing attention. Therapeutic and preventive supplementations with vitamins and minerals have been used successfully for a long time for various clinical conditions (Singhai & Austin, 2002). These include vitamin A for maintenance of vision, beta-carotene in erythropoietic protoporphyria, vitamin C in scurvy and niacin in pellagra. *In vitro* and animal studies have shown the immuno-stimulatory and anti-cancer properties of several micronutrients, leading to large epidemiological trials of micronutrient supplementation (Bijlsma, 2001; Singhai & Austin, 2002). Correction of the nutritional imbalance in HIV infection is being recognized as an important part of the comprehensive care of persons infected with HIV.

Evidence from different studies (C[illegible] 995 and 1997; Allard *et al.*, 1998; Coutsoudis *et al.*, 1999; Kanter *et al.*, 1999; Bouic *et al.*, 2001) continues to accumulate, showing that carefully chosen supplementation may help in enhancing a better quality of life.

Micronutrient supplementation has been advocated for HIV-infected persons, especially in low-income countries such as South Africa (Van Staden *et al.*, 1998; Kennedy *et al.*, 2000; Singhai & Austin 2002). Supplementation has been shown to be associated with a significant slowing of HIV disease progression, better preservation of $CD4^{+}$T-cell counts and lower viral loads (Dworkin, 1994; Fawzi *et al.*, 2004). There is evidence that nutritional intervention assists in maintaining and optimising nutritional status and immune function; prevents the development of nutritional deficiencies, loss of weight and lean body mass; promotes the response to medical treatment, and increases longevity in HIV-infected persons (Coodley, et *al.*, 1996; Fawzi & Hunter, 1998; Fawzi *et al.*, 1999; 2000; Piwoz & Preble, 2000; Willsumsen, 2003; Oguntibeju *et al.*, 2003; Fawzi, 2003).

It has been further demonstrated by Bijlsma (2001) that micronutrient supplementation may be an important prophylactic and therapeutic measure for HIV-1 infected persons and is possibly one of the few potential therapies that could improve the patient's quality of life by maintaining strength, comfort, level of functioning and self-image. For example, daily β-carotene supplements of 180 mg provided for HIV-positive persons for one month resulted in a small but statistically significant increase in total white blood cell count and a percentage change in $CD4^{+}$ T-cell count in one trial (Coodley *et al.*, 1993; 1996). In another study, Fawzi *et al.* (1999) reported that supplementation with multivitamins resulted in a significant increase in the number of $CD4^{+}$T-cell counts and $CD8^{+}$T-cell counts. In the same study, haemoglobin levels were significantly increased.

In a clinical trial conducted by Allard *et al.* (1998) in Canada, it was observed that daily mega doses of vitamins C and E intake provided for three months resulted in a clinically important reduction in viral load. In 1998, Lamprecht and his co-workers published two pilot studies on cats infected with FIV (feline immunodeficiency virus), the feline equivalent of HIV. In both studies, the cats that received a mixture of sterol and sterolins (100:1) maintained stable $CD4^{+}$T-lymphocyte counts and suffered no death due to FIV within three years. These results prompted an open trial of 80 human patients with HIV over a three-year period with clinical monitoring [illegible]ns. The average $CD4^{+}$T lymphocyte counts remained stable for three years with no significant declines, which is similar to the results obtained with the experimental cats. There was also a decrease in average viral load (Bouic *et al.*, 2001). Kanter *et al.* (1999), in a study in South Africa found that a daily vitamin B-complex and multivitamin intake delayed the onset of AIDS and death. However, studies on nutritional supplementation in HIV positive persons are still scarce in Africa and South Africa (Muslimatun *et al.*, 2001).

Researchers share the view that as the virus infects and selectively attacks and depletes T-lymphocytes bearing the CD4 receptor (T-helper cells) in the host, it causes a predisposition to opportunistic infections and malignancies (Wilson *et al.*, 2000; SAHIVCS, 2001), and the $CD4^{+}$T-cell count is noted to provide prognostic information, diagnose immunological AIDS and determine when prophylactic treatment is needed. Therefore, the $CD4^{+}$T-cell count remains an important measure of the status of the immune system and it is still used to make important clinical decisions regarding prophylaxis for a number of opportunistic infections. For instance, a $CD4^{+}$T-cell count of < 300-350 cell/mm^3 may warrant anti-tuberculosis treatment to be considered while a $CD4^{+}$T-cell count of < 200 cells/mm^3 might be an indication for prophylaxis against *Pneumocystis carinii* (Martin, 2000).

The entire natural history of HIV infection from transmission to death can most easily be described in terms of various disease stages, each of which relates to particular levels of immune system functionality (SAHIVCS, 2001). At present, HIV infection is commonly divided into stages, predominantly on the basis of $CD4^{+}$T-cell counts and the most common clinical symptoms, but also on the basis of rates of viral replication. Martin (2000) indicated that the most striking laboratory feature of acute sero-conversion is marked lymphopaenia, with depletion of $CD4^{+}$T and $CD8^{+}$T-lymphocytes. According to Martin (2000), this is often followed by a period of relative lymphocytosis (mostly $CD8^{+}$T-lymphocytes), with a high proportion of atypical lymphocytes. Close to the time of resolution of primary infection symptoms, the $CD4^{+}$T-cell count increases, but rarely returns to baseline, while the normal $CD4^{+}/CD8^{+}$ ratio is reversed (Martin, 2000).

It has been shown that early-stage disease can be defined by a $CD4^{+}$T-cell count of > 500 cells/mm^3, corresponding to the World Health Organization (WHO) clinical stages 1 and 2 and the only consistent physical ex with this stage is generalised lymphadenopathy (WHO, 1993; UNAIDS, 2002). There is evidence that the intermediate stage of HIV disease can be defined immunologically as that occurring in persons with a $CD4^{+}$T-cell count of 200-500 cells/mm^3, or clinically by the presence of WHO stage 3 diagnoses, while late-stage and advanced stages can be defined by $CD4^{+}$T-cell counts of 50-200 and < 50 cells/mm^3 respectively (Martin, 2000), with a high risk of developing opportunistic infections and wasting.

On the other hand, viral load can independently predict the rate of progression of immune suppression and is the most meaningful tool for determining response to anti-retroviral therapy (Coombs *et al.*, 1996; Martin, 2000). In one study, each two-fold decrease in the viral load level during treatment correlated with a 27% reduction in the relative hazard of disease progression (Coombs *et al.*, 1996). It has been reported that each three-fold decrease in the viral load level is associated with a 63% reduction in the relative hazard of progression (O,Brien *et al.*, 1996). It is believed that factors such as genetic susceptibility, viral load, concurrent infections, as well as the pre-existing immune status at the time of HIV infection, determine the duration of clinical latency to progression to AIDS (Piwoz & Preble, 2000; Semba & Tang, 1999; Fawzi, 2003). Most research findings support the view that the plasma viraemia is very high during acute sero-conversion, while circulating viral particles remain detectable throughout all stages of the disease, unless treatment is provided with highly active antiretroviral therapy (Clark & Shaw, 1993; Bouic *et al.*, 2001).

From previous studies, researchers have expressed the view that further and urgent community-based research studies are needed on nutritional supplementation among HIV-positive/AIDS individuals. For instance, Van Staden *et al.* (1998) reported deficiencies in several micronutrients in HIV-1 sero-positive patients in the Free State province of South Africa and suggested a multi-vitamin/anti-oxidant supplementation to improve the immune status in these patients. In the developing countries where a majority of the people cannot afford antiretroviral therapy, nutrition combined with supplementation could form a good source of therapy. Although nutritional supplementation cannot single-handedly reverse a severely depleted immune system, nutritional supplementation could improve the immune function, nutritional status and general well-being of HIV-positive/AIDS patients.
As the HIV/AIDS pandemic enters its third decade and case numbers continue to increase, neither have definitive cures been found nor has an effective vaccine been developed. It

is therefore time to examine othe[illegible] reducing the HIV infection burden. Nutritional intervention may be one such an approach, particularly in the Free State Province of South Africa where the effect of nutritional supplement on the immune status of HIV-positive/AIDS patients has not been examined.

It is against this background that the influence of a nutritional supplement on the immune status, haematological parameters and clinical conditions of HIV-positive/AIDS patients was examined.

6.3 Materials and Methods

This was an open-labelled, multiple-dose clinical trial consisting of 50 HIV-positive /AIDS volunteers. Of the 50 volunteers that met the inclusion criteria and were recruited into the study, 3 dropped out, 10 died, while 2 relocated to other towns during the three months period. Of the 35 patients that completed the food frequency questionnaire and had their anthropometric parameters measured at baseline, only 29 completed this section of the study (blood parameters measurement). The dietary intakes and anthropometric profile of the patients are reported in chapters 4 and 5 respectively. An approval from the Ethics Committee (ETOVS 32/03) of the Faculty of Health Sciences, University of the Free State was obtained and all patients signed informed consent forms for inclusion in the study. Inclusion criteria include: male and female patients between 18 to 65 years of age that are HIV-positive, not on antiretroviral therapy, are willing to undergo a pre-and post-study physical and medical examination, are found to be within the range of clinical acceptability in medical history and physical examination, have a $CD4^{+}$T-cell count of 100-350 cells/mm^3 and are able to comprehend and willing to sign the statement of informed consent. Only the patients that met the inclusion criteria were included in this study.

The study was conducted in two separate clinics, namely Tsepo House (home based care) and Medi Inn clinic in Bloemfontein. The patients seen at Medi Inn clinic were patients from the South African Red Cross home-based care, Bloemfontein, and transported to the Medi Inn clinic on arrangement and in agreement with the Red Cross management with the consent of the patients. The study involved a baseline visit and 3-monthly visits to Medi Inn clinic and Tsepo House (home based care) from April to September 2003 (at these two clinics, the patients were seen, signed the consent form, were examined medically, completed the food freque e with their anthropometric values determined and had blood samples drawn for analysis). The duration of this study is similar to the one reported on by Allard *et al.* (1998).

6.3.1 Sample Collection and Processing

Ten ml (10 ml) of blood was collected from each patient by a registered health professional into EDTA sampling tubes for the determination of full blood count, $CD4^{+}$T-cell count and viral load. Blood samples were kept at room temperature and processed within 4 hours. Haematological parameters were performed on all patients using a Beckman cell Coulter (USA).

The patients' T-cell subset numbers were determined by using a Becton Dickinson Multi TEST CD3 FITC/CD8 PE/CD45 Per CP/CD4 APC, four-colour direct immuno-fluorescence monoclonal antibodies with a suitably equipped flow cytometer (Coulter. Hialeah. FL. U.S.A) to identify and determine the counts of mature human T-lymphocytes in lysed whole blood.

A quantitative HIV-1 RNA polymerase chain reaction (PCR) was performed on batched samples using an HIV-1 monitor assay according to the manufacturer's instructions (AMPLICOR HIV-1 Monitor Test Roche Molecular Systems. Inc Somerville, N.J., U.S.A) for the detection and quantitation of the viral loads. In each case, 50µl of each prepared RNA sample was used for PCR. Following amplification and detection of the PCR product, the starting HIV-1 RNA load in each sample was calculated in accordance with the internal quantitation standard (QS), with results expressed as HIV-1 RNA copies/ml plasma (Romeu *et al*, 1992). The above-mentioned parameters were determined at baseline and after supplementation. The patients' HIV status were already known as evidenced by previous laboratory records, hence they were not determined in this study.

6.3.2 Supplementation of patients

Following the determination of the baseline dietary intake, anthropometry profile, haematological and immunological parameters and viral loads, the patients were given 7.5 ml of the test supplement twice daily (between 07:00-09:00 and 16:00-19:00 hours). The medically, completed the food freq[illegible]ire with their anthropometric values determined and had blood samples drawn for analysis). The duration of this study is similar to the one reported on by Allard *et al.* (1998).

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6.3.3 Follow-up Visits

The patients visited the clinic on a monthly basis. On each visit, physical and medical examinations were carried out on each patient by the clinical investigator in the study. Blood samples were taken and the full blood count and $CD4^+$/$CD8^+$T-cell count were determined at the monthly visit while the viral load was repeated at the end of the study. The haematological and immunological parameters were repeated at the end of the study to determine the possible influence of the supplement on the haematological and immunological status of the patients. For detailed procedure, see chapter 3 (methodology **3.4.2**). Compliance with the regime was ensured by counting the supplement units on a daily basis and at each visit and by constantly reminding the patients of the need to follow the protocol. Compliance with supplement intake by patients was monitored by assistants of the Red Cross home-based care, Bloemfontein.

6.4 Statistical analysis

The results for this study were analysed by an independent Biostatistician at the University of the Free State, Bloemfontein, South Africa, using SAS (1990). A detailed description is contained in chapter 3 (see **3.5.7**)

6.5 Results

The results indicate that the female patients (n=27) were significantly more ($P<0.05$) than the male (n=8) patients. The results in some cases were categorised according to gender. Table 6. 1 shows the mean, standard deviation, median and the p-value of haematological parameters of the whole group of HIV-positive/AIDS patients at baseline and final visit. Haematological parameters with the same superscript showed statistical significance ($P<0.05$). Symbols and what they represent are indicated below each of the tables which follow.

Table 6.1: Haematological parar hole group of HIV-positive/AIDS patients at baseline and final visit.

Variables	Baseline visit N=35			Final visit N=29			P-value
	Mean	SD	Median	Mean	SD	Med	
RBC: x10^9/l	4.0	0.6	4.1	4.4	1.7	4.0	NS
Haemoglobin: g/dl	12.1	1.9	12.0	11.9	1.7	11.9	NS
Hct: l/l	0.37	0.1	0.36	0.38	0.1	0.36	NS
MCHC: g/dl	32.8	0.9	33	33.6	1.0	34	<0.0002*
ESR: mm/hr	83.7	32.8	86	86.7	24.1	83	NS
MCV: fl	91.8	5	92	87.7	4.9	90	<0.002*
MCH: pg	30.2	5	31	30.1	2.4	30	NS
RDW	15.1	1.5	14.	15.2	2.0	14.5	NS
WCC: x10^9/l	5.7	3.9	4.9	4.8	1.6	4.8	NS
Neutrophil: x10^9/l	2.7	2.6	2.2	2.2	1.0	2.2	NS
Lymphocyte: x10^9/l	2.1	0.8	1.9	1.9	0.9	1.9	NS
Monocyte: x10^9/l	0.6	0.4	0.5	0.4	0.3	0.4	<0.001*
Eosinophil: x10^9/l	0.3	0.4	0.2	0.3	0.3	0.2	NS
Basophil: x10^9/l	0.1	0.5	0.02	0.03	0.02	0.02	NS
Platelets count: x10^9/l	254.3	82.3	252	239.6	74.4	234	NS

The P value tested the difference between the haematological parameters at baseline and final visit.

*: Parameters with the same superscript showed statistical significance (P<0.05).

NS (non significant), Med (median), SD (standard deviation). RBC (red blood cell), Hct (Haematocrit), MCV (mean cell volume), MCH (mean cell haemoglobin), MCHC (mean cell haemoglobin concentration), RDW (red cell distribution width).

Table 6.2 shows haematological p[illegible]seline and final visits according to gender.

Table 6.2: Haematological parameters of HIV-positive/AIDS patients at baseline and final visits according to gender.

Variables	Baseline visit N=35			Final visit N=29		
	Mean	SD	P value	Mean	SD	P value
RBC: x10^9/l						
Male	4.4	0.8	0>0.05	4.4	2.0	0>0.05
Female	3.9	0.6		4.4	2.0	
Haemoglobin: g/dl						
Male	13.7	2.5	<0.03*	13.4	2.6	0.1
Female	11.6	1.5		11.4	3.1	
Hct: l/l						
Male	0.41	0.1	<0.02*	0.39	0.1	0>0.05
Female	0.36	0.04		0.38	0.1	
MCHC: g/dl						
Male	33.5	0.9	<0.05*	33.8	1.3	0>0.05
Female	32.1	0.9		33.5	0.9	
ESR: mm/hr						
Male	95.9	24.9	0>0.05	87.4	18.8	0>0.05
Female	80.1	34.4		86.4	26.2	
MCV: fl						
Male	90.8	4.3	0>.05	92.8	4.6	<0.05*
Female	89.2	3.9		91.6	4.9	
MCH: pg						
Male	33.5	2.2	<0.01*	32.5	1.8	<0.05*
Female	30.0	1.9		29.1	2.1	
RDW						
Male	15.6	1.7	>0.05	13.1	2.0	0>0.05
Female	16.0	2.3		15.7	2.1	
WCC: x10^9/l						
Male	5.9	3.8	0>0.05	4.8	1.6	0>0.05
Female	4.8	2.7		4.4	1.3	
Neutrophil: x10^9/l						
Male	2.9	2.6	0>0.05	3.0	3.2	0>0.05
Female	2.4	1.9		2.3	2.6	
Lymphocyte: x10^9/l						
Male	2.3	0.8	0>.05	2.4	0.9	0>0.05
Female	1.8	0.7		2.1	0.9	
Monocyte: x10^9/l						
Male	0.6	0.4	0>0.05	0.5	0.1	0>0.05
Female	0.7	0.5		0.6	0.3	
Eosinophil: x10^9/l						
Male	0.3	0.2	0>0.05	0.3	0.3	0>0.05
Female	0.4	0.3		0.2	0.2	
Basophil: x10^9/l						
Male	0.1	0.5	0>0.05	0.02	0.01	0>0.05
Female	0.1	0.5		0.03	0.02	
Platelets count: x10^9/l						
Male	258.5	83.1	0>0.05	241.2	76.1	0>0.05
Female	250.1	79.7		238	74.7	

*<0.05 (significant)

>0.05 (insignificant)

Table 6.4: Immunological param sitive/AIDS patients according to gender.

Variables	Baseline visit N=35			Final visit N=29		
	Mean	SD	P value	Mean	SD	P value
Total T-cell count/mm^3						
Male	1617	737.1	0>0.05	1543	697	0>0.05
Female	1498	746.3		1344	701.9	
CD4$^+$T-cell count/mm^3						
Male	204.2	83.8	0>0.05	188.6	86.2	0>0.05
Female	202.4	81.2		182.2	90.1	
CD8$^+$T-cell count/mm^3						
Male	1411	671.3	<0.02*	1255	675.3	0>0.05
Female	1390	686.5		1247	668.4	
CD4/CD8 ratio						
Male	0.16	0.1	0>0.05	0.18	0.1	0>0.05
Female	0.18	0.1		0.17	0.1	
Viral load/ml						
Male	374308	300301	0>0.05	279371	244876.2	0>0.05
Female	374304	300304		279373	244878.1	

*: P<0.05 (significant).

P>0.05 (insignificant)

Table 6.4 shows the immunological parameters of HIV-positive/AIDS patients according to gender. The CD8$^+$T-cell count was significant (P<0.02) at baseline between male and female. Other parameters as shown in table 6.4 were not significant (P>0.05) between male and female at baseline and final visit. The supplement tends to indicate a positive effect on the CD8$^+$T-cell count according to gender but was not significant.

In Table 6.5, ten (28.6%) of the pat ction at baseline and this decreased to 5 (17.2%) following treatment and supplementation. Eight (22.9%) of the patients had candidiasis at baseline and this decreased to 5 (17.2% with supplement administration. Six of the patients (17.1%) had tuberculosis (TB) at baseline. At the end of the study, there was no incidence of TB reported. Three of the patients (8.6%) had diarrhoea at baseline visit, which decreased to 2 (6.9%) by the end of study. Liver enlargement was observed in 7 of the patients and it persisted through the study. Skin rashes decreased from 10 (28.6%) at baseline visit to 3 (10.3%) by the end of study.

Most of the patients had low blood pressure and a greater percentage had a normal pulse rate. Overall, the patients showed slight improvements in clinical condition as well as physical appearance and quality of life.

Table 6.5: Physical and Clinical Conditions of HIV-positive/AIDS Patients before and after nutrient supplementation.

System	Baseline visit n=35	V1 n=30	V2 n=32	V3 n=29
1. Eyes				
▪ Jaundice	0	0	0	0
▪ Discharge	0	0	0	0
▪ Infection	1 (2.9%)	1 (2.9%)	0	0
▪ Pupil Normal	29 (82.9%)	29 (96.7%)	29 (90.6%)	29 (100%)
2. Ears				
▪ Pain	6 (17.1%)	4 (13.3%)	3 (9.4%)	2 (6.9%)
▪ Infection	10 (28.6%)	8 (26.7%)	6 (18.8%)	5 (17.2%)
▪ Discharge	7 (20%)	6 (20%)	5 (15.6%)	4 (13.8%)
3. Nose				
▪ Pain	0	0	0	0
▪ Ulcer	0	0	0	0
▪ Swelling	2 (5.7%)	0	1 (3.1%)	1 (3.4%)
4. Mouth & Lips				
▪ Pain	1 (2.9%	0	0	0
▪ Ulcer	6 (17.1%)	5 (16.7%)	3 (9.4%)	1 (3.4%)
▪ Swelling	1 (2.9%)	2 (6.7%)	2 (6.3%)	2 (6.9%)
▪ Cracked lips	2 (5.7%)	2 (6.7%)	2 (6.3%)	1 (3.4%)
▪ Candidiasis	8 (22.9%)	7 (23.3%)	6 (18.8%)	5 (17.2%)
5. Throat				
▪ Pain	15 (46.9%)	12 (40%)	15 (46.9%)	13 (44.8%)
▪ Ulcer	3 (8.6%)	2 (6.7%)	2 (6.3%)	4 (13.8%)
▪ Infection	21 (60%)	20 (66.7%)	21 (65.6%)	16 (55.2%)
▪ Candidiasis	3 (8.6%)	4 (13.3%)	5 (15.6%)	5 (17.2%)
6. Respiratory System				
▪ Cough	13 (37.1%)	21 (70%)	17 (53.1%)	14 (48.3%)
▪ Shortness of breath/Dyspnoea	3 (8.6%)	3 (10%)	3 (9.4%)	1 (3.4%)
▪ Pain	7 (20%)	12 (40%)	9 (28.1%)	10 (34.5%)
▪ Haemoptysis	0	0	0	0
▪ TB	6 (17.1%)	5 (16.7%)	2 (6.9%)	0
▪ Abnormal breath sounds	5 (14.3%)	13 (43.3%)	13 (40.6%)	11 (37.9%)

V1 (visit one), V2 (visit two), V3 (visit three).

Table 6.5 (continued): Physica Conditions of HIV-positive/AIDS Patients before and after Nutrient Supplementation.

7. Neck				
▪ Swelling	2 (5.7%)	2 (6.7%)	1 (3.1%)	1 (3.4%)
▪ Glands:				
Submental	5 (14.3%)	6 (20%)	4 (12.5%)	1 (3.4%)
Jugular	13 (37.1%)	20 (66.7%)	18 (56.3%)	15 (51.7%)
Posterior Triangle of the Neck	5 (14.3%)	7 (23.3%)	6 (18.8%)	5 (17.2%)
Occipital	16 (45.7%)	17 (56.7%)	15 (46.9%)	11 (37.9%)
8. Cardiovascular				
▪ Swelling of legs/angles	2 (5.7%)	0	4 12.5%)	1 (3.4%)
▪ Other Edema	1 (2.9%)	0	4 (12.5%)	1 (3.4%)
▪ Shortness of breath/Dyspnoea	4 (11.4%)	5 (16.7%)	3 (9.4%)	3 (10.3%)
▪ Cyanotic	0	0	1 (3.1%)	0
▪ Club fingers	0	0	0	0
9. Blood forming organs				
▪ Enlargement of spleen	0	0	0	0
▪ Glands in the neck	17 (48.6%)	20 (66.7%)	18 (56.3)	16 (55.2%)
▪ Axilla	11 (31.4%)	17 (56.7%)	10 (31.3%)	11 (37.9%)
▪ Groins	10 (28.6%)	13 (43.3%)	9 (28.1%)	10 (34.5%)
10. Digestive				
▪ Weight loss/Malnutrition	20 (57.1%)	16 (53.3%)	11 (34.4%)	10 (34.5%)
▪ Eating well	17 (48.8%)	15 (50%)	17 (53.1%)	18 (62.1%)
▪ Normal bowel movement	26 (74.3%)	26 (86.7%)	28 (87.5%)	28 (96.6%)
▪ Diarrhoea	3 (8.6%)	3 (10%)	2 (6.3%)	2 (6.9%)
▪ Constipation	1 (2.9%)	1 (3.3%)	1 (3.1%)	1 (3.4%)
▪ Abnormal pain	2 (5.7%)	0	0	0
▪ Nausea/Vomiting	0	0	0	0
▪ Liver enlargement	7 (20%)	7 (23.3%)	7 (21.9%)	7 (24.1%)
▪ Spleen enlargement	0	0	0	0
11. Genito-urinary				
▪ Dysuria	6 (17.1%)	4 (13.3%)	4 (12.5%)	4 (13.8%)
▪ Dysmenorrhoea	2 (5.7%)	1 (3.3)	2 (6.3%)	1 (3.4%)
▪ Vaginal discharge	6 (17.1)	11 (36.7%)	11 (34.5%)	10 (34.5%)
▪ Urethral discharge	0	0	0	0
▪ STI	2 (5.7%)	2 (6.7%)	2 (6.3%)	2 (6.9%)
▪ Penile discharge	0	0	0	0
▪ Swelling of scrotum	0	0	0	0
12. Locomotor				
▪ Pain	5 (14.3%)	5 (16.7%)	8 (25%)	3 (10.3%)
▪ Neuropathy	10 (28.6%)	12 (40%)	7 (21.9%)	9 (31%)
13. Skin				
▪ Rashes	10 (28.6%)	9 (30%)	8 (25%)	3 (10.3%)
▪ Ulcers	4 (11.4%)	5 (16.7%)	4 (12.5%)	4 (13.8%)
▪ Tumours	0	0	0	0
14. Central Nervous				
▪ Paralysis	0	0	0	0
▪ Normal state of consciousness	32 (91.4%)	30 (100%)	29 (90.6%)	25 (86.2%)
▪ Normal sensory system	32 (91.4%)	30 (100%)	30 (93.8%)	29 (100%)
15. Vital Signs				
▪ Temperature/Normal	31 (88.6%)	30 (100%)	31 (96.9%)	29 (100%)
▪ Blood pressure/Normal	7 (20%)	3 (10%)	5 (15.6%)	7 (24.1%)
▪ Pulse Rate/Normal	21 (60%)	21 (70%)	18 (56.3%	17 (58.6%)

V1 (visit one), V2 (visit two), V3 (visit three).

6.6 Discussion

From available information, the current study is the first clinical trial in the Free State Province of South Africa and the results demonstrated that in an HIV-positive population, daily supplementation of sterol/sterolin mixture and antioxidants significantly decreased the viral load. Researchers have recommended the benefit of nutrition intervention programmes, including supplementation with antioxidant nutrients, as part of the comprehensive care for HIV-positive/AIDS people (Bogden *et al*., 1990; Fawzi & Hunter, 1998; Miller & Gorbach, 1999). In this study, the blood levels of these antioxidants were not determined because it was not the primary focus; in addition, there were financial constrains. However, a previous study done in the Free State by Van Staden *et al.* (1998) reported reduced levels of these antioxidants in the blood of HIV-positive persons. It was therefore appropriate to test the influence of a multiple combination liquid product containing sterol/sterolin and antioxidants on the immune status, haematological parameters and clinical conditions of HIV-positive/AIDS patients. It is believed that an extra amount of antioxidants would help in reducing the stimulation of HIV replication and thus have some clinical significance (Bogden *et al*., 1990)

In vitro evidence has implicated oxidative stress in the stimulation of HIV replication through activation of the necrosis factor in a human T-cell line (Das *et al*., 1990). Addition of antioxidant vitamins blocked the activation of the necrosis factor and inhibited HIV replication (Das *et al*., 1990; Harakeh *et al*., 1990; Wong *et al*., 1991). Similar effects may occur *in vivo* as demonstrated by the significant reduction in HIV viral load in both male and female patients, as observed in this study following supplementation. This finding is similar to that reported by Allard *et al.* (1998) who observed that, in a clinical trial, daily doses of vitamin C and E supplementation provided for patients for three months resulted in a clinically important reduction in the viral load. In another study, Bouic *et al.* (2001) indicated that HIV-positive subjects on a sterol/sterolin mixture experienced a decrease in average viral load in their plasma. The supplement in the present study contains sterol/sterolin mixture besides antioxidants/minerals. The question as to whether the significant reduction in the viral load was due to the effect of the sterol/sterolin mixture or a combined effect of the sterol/sterolin mixture and the other supplement components (antioxidants/minerals), needs to be clarified in future studies. Yet, important questions are whether the reduction observed regarding the viral load can be sustained for an appreciable period of time and whe[...]y demonstrates clinical benefits. It is likely that the clinical benefits of the supplement are related to duration as well as to the magnitude of HIV suppression, but the precise duration of HIV suppression necessary to effect measurable clinical benefits needs to be clearly defined. A mere observation in the reduction of the HIV RNA (viral load) may be inadequate in measuring the prospect of a clinical benefit of a supplement. For instance, one log decrease for an individual with a viral load of 100, 000 copies may be more beneficial to that individual than one log reduction for an individual with 10, 000 RNA copies. Likewise, a one log reduction for an individual with 100, 000 RNA copies will lower his or her viral load to 10, 000 copies, and one log reduction for an individual with 10, 000 RNA copies will lower the viral load to 1000 RNA copies. Nonetheless, the result obtained from the study is envisaged to stimulate further work on the potential use of this supplement in HIV therapy.

Kanter *et al.* (1999) reported delayed onset of AIDS and death following supplementation with multivitamins. The current study did not directly measure the rate of progression of HIV infection to AIDS or the survival rate; however, evidence in literature (Martin, 2000; Piwoz & Preble, 2000; Singhai & Austin, 2002) showed that one of the factors determining disease progression in HIV infection is the rate of replication of the HIV. By decreasing the viral load significantly as observed in this study, the supplement has indirectly shown its positive effect on the quality of life of people living with HIV/AIDS, which relates to reducing the rate of disease progression.

It has been indicated that nutrient supplementation may be an important prophylactic and therapeutic means for HIV-positive people to improve their quality of life and level of functionality (Piwoz & Preble, 2000; Macallan, 1999; Allard *et al.*, 1998). Decreasing the viral load is a scientifically accepted means of improving the quality, strength and level of such functionality since this boosts the immune status. The level of functionality and quality of life that emerged from the influence of the supplement in this study cannot be categorically stated, but by inference, it can be said that the supplement improved the quality of life by decreasing the progression of HIV infection.

A central feature in the pathogenesis of HIV infection is the depletion of T-lymphocytes bearing the $CD4^+$ molecule (Rowland-Jones & Pinheiro, 2001). The $CD4^+$T-lymphocyte quantitation measures this depletion and provides important information about the immune status of HIV-1 infected persons, the $CD4^{+}$T-cell count decreased significantly during the course of study. This does not infer that the supplement further decreased the $CD4^{+}$T-cell counts, but the decrease in the $CD4^{+}$T count reflects the severe state of the immune status. It was expected that, with a decrease in viral load, the $CD4^{+}$T count would increase. This was not so, as can be seen in the study. The quantity and function of $CD4^{+}$T-cells is dependent on the production of new $CD4^{+}$T-cells. If production of these cells is impaired, damaged $CD4^{+}$T-cells cannot be replaced, therefore a decrease in viral load may not necessarily result in a corresponding increase in the $CD4^{+}$T count. $CD4^{+}$T-cell depletion in HIV-1 infection is partly the result of T-cell apoptosis (Hansjee *et al.*, 2004). According to Hansjee *et al.* (2004), the significant relationship between residual T-lymphocyte apoptosis and $CD4^{+}$T-cell recovery suggests that persistent apoptosis may impede immune restoration. Although T-lymphocyte apoptosis was not investigated in this study, it is possible that it contributed partly to the non-significant effect of the supplement on the $CD4^{+}$T-cell count. It has also been observed that suppression of plasma viraemia in chronic HIV infection is usually associated with loss or reduction of HIV-1 specific cytotoxic T-lymphocyte responses (Altfeld & Rosenberg, 2000). Could this be a possible mechanism that contributed in a secondary way to the reduction of the $CD4^{+}$Tcell count of the studied patients? Although it is not clear whether it was so, further investigation could throw more light on this.

In addition, researchers shared the view that nutritional intervention, including supplementation, should commence in the early stage of HIV infection. Both clinical and laboratory data suggest that the majority of the patients recruited for this study were not in an early stage of HIV infection. Some findings have reported that the measure of $CD4^{+}$T count could be misleading in predicting HIV progression compared with viral load (Mellors *et al.*, 1996; Wilson *et al.*, 2000; Rowland-Jones & Pinheiro, 2001), therefore viral load seems a better predictor of HIV replication and the rate of decline of the immune status in HIV-infected persons. The viral load tells us with remarkable precision how fast the immune system is being destroyed. Martin (2000) indicated that $CD4^{+}$ and $CD8^{+}$T-cell counts decrease as HIV infection progresses in the absence of highly active antiretroviral therapy (HAART). Martin's finding (2000) could partly explain the reason for the decline in the $CD4^{+}$T-cell count observed in the study. Time of collection and analysis of blood sample could be factors as well. It is recommended that blood samples for a $CD4^{+}$T-cell count be collected between 09:00 and 11:00 and analysed within four hours of blood collection. In this study, this was n cause patients could not all arrive at the clinic at the same time for sample collection.

In a study carried out in South Africa by Bouic *et al.* (2001), the CD4^{+}T-cell count remained stable for three years with no significant increase or decrease following supplementation. The product/supplement used by Bouic *et al.* (2001) contained mainly sterol and sterolin (an immune modulator), but no antioxidants. The supplement examined in our study contains sterol and sterolin mixture but in addition, also contains antioxidants. However, it showed no stability or increase in the CD4^{+}T-cell count. The reason for the difference in result may be related to the stage of HIV infection and duration of administration of the supplement. One study carried out in Canada by Allard *et al.* (1998), reported no significant difference between the baseline CD4^{+}T-cell count and the CD4^{+}T-cell count at the end of three months of supplementation. The combination of the supplement (in his case, it was vitamin E and C), the stage of infection, and the bioavailability of supplement components in the individual subjects, may explain the difference in the CD4^{+}T-cell count at baseline and the CD4^{+}T-cell count at the end of study.

Although AIDS-defining complications are common once the CD4^{+}T-cell count is less than 200 cells/ml, the association between the CD4^{+}T-cell count and the risk of death in persons with AIDS is not well defined. In this study, ten of the patients whose CD4^{+}T-cell count fell below 200 cells/mm^{3} died during the course of the study. However, a few of the patients who had a CD4^{+}T-cell of less than 200 cells/mm^{3} survived till the end of the study. Therefore, this current work tends to suggest that this population somehow constituted a select group who survived to this point with such low levels of CD4^{+}T-cell count.

The issue of non-compliance as regards the influence of the supplement on the CD4^{+}T-cell count in this study cannot be justified. This is because it could be argued that since the supplement significantly reduced the viral load, the patients possibly complied with the supplement regime. But, at the same time, it might be said that the compliance that produced a significant decrease in the viral load may not necessarily produce a positive effect on the CD4^{+}T-cell count; a better compliance would probably be required to possibly produce a positive effect on the CD4^{+}T-cell count. Other reasons for the non-positive effect of the supplement on the CD4^{+}T-cell count observed in this study are suggested to be the bioavailability of the supplement ingredients and drug/food-nutrient interaction.

The $CD8^+$ T-lymphocytes are beli[illegible] antiviral activity against HIV (Yang, 1998). This was first reported in 1986, when investigators noted that depletion of $CD8^+$T-cells from peripheral blood mononuclear cells of HIV-infected persons resulted in a marked increase in viral replication. Replacement of the $CD8^+$T-cells caused a dose-dependent suppression of viral replication (Yang, 1998). Yang (1998) also reported that an increase in the $CD8^+$T-cells count correlated with a decline of viraemia of primary HIV infection and inversely correlated with disease progression. In this study, the $CD8^+$T cells showed no correlation with decrease in viral load, probably because the $CD8^+$T-cell count did not increase with supplementation. It could be opined that, if the $CD8^+$T-cells count had increased significantly following supplementation, a correlation of $CD8^+$T-cell counts with viral load could be possible. The mechanism as to how increased $CD8^+$T cells decrease viral activity is not clear, although this mechanism was not examined in this study. However, it is believed that $CD8^+$T-cells may produce factors that act by inhibiting the promoter, the long terminal repeat of HIV, thereby suppressing viral transcription (Yang, 1998).

HIV infection is known to cause inversion of the $CD4^+$T-cell/$CD8^+$T-cell ratio (normal range 0.72-3.14). A $CD4^+$/$CD8^+$T-cell ratio of 0.15 is said to correlate with a $CD4^+$T-cell count of 200cells/mm^3. A high baseline $CD8^+$T-cell count and subsequent fall seems to occur due to HIV-mediated immune hyper-activation (Wilson *et al.*, 2000). The median $CD4^+$/$CD8^+$T-cell count ratio of 0.2 reported in this study, is similar to the 0.15 quoted above and tends to correlate with the level of the $CD4^+$T-cell count. The present study also indicated that the $CD4^+$/$CD8^+$T-cell count ratio was reversed. The reversed ratio is believed to be associated with the significant decline in the $CD4^+$T-cell count.

The haemoglobin level stayed relatively constant in the patients Anaemia here is defined as haemoglobin below 12.0 g/dl (table 6.1). The haemoglobin level in all the patients was slightly above 12.0 g/dl at baseline and this level was more or less constant throughout the course of the study. It is possible that haemo-concentration contributed to the haemoglobin level reported in this population. When the patients were examined according to gender, there was a slight but insignificant decrease in the haemoglobin level of female patients following supplementation. The mean cell haemoglobin (MCH) level was also slightly maintained. The haematocrit (Hct) showed a slight but an insignificant increase following supplementation (table 6.1). The mean cell volume (MCV) and the mean cell haemoglobin concentr owed significant increase following supplementation (table 6.1).

Anaemia is a common manifestation of HIV infection and occurs at all stages of HIV-associated disease (Doukas, 1998). It remains a significant problem despite recent advances in antiretroviral therapy and the introduction of highly antiretroviral therapy (HAART) into routine clinical practice (Moore, 2000). Among HIV-infected patients, anaemia has been linked to decreasing levels of CD4$^+$T-cell counts (more severe with CD4$^+$T-cell count less than 200), increased plasma HIV RNA levels, and a history of clinical AIDS-defining conditions. Anaemia in HIV-infected persons has also been linked to abnormally low levels of erythropoietin, decreased erythropoietin production, increased erythrocyte destruction, ineffective erythrocyte production and the use of multiple medications (Doukas, 1998)

Taken as a group, the patients had haemoglobin levels slightly above 12.0 g/dl (table 6.1) at baseline, hence the patients cannot be said to be anaemic. With the progression of HIV infection, the level of haemoglobin in HIV infected patients is expected to decrease in the absence of nutritional support. In this study, it was observed that, instead of the haemoglobin of the patients having decreased, the level was maintained through the course of the study. We tend to believe that the haemoglobin level was maintained in the presence of the supplement. The supplement contains important ingredients such as folic acid, vitamin C and vitamin B_{12} that could have possibly contributed to maintaining the haemoglobin level. Folic acid is essential in the formation and maturation of red blood cells (Itam, 2003). Vitamin C is a reducing agent and its effect is to prevent the oxidation of iron, thus making more iron available. The effect of vitamin B_{12} in red blood cell synthesis is well known. In the presence of adequate amounts of vitamin B_{12} (10µg), it is believed that megaloblastic anaemia of vitamin B_{12} origin can be prevented (Chemiron International, 1991). Although the observation may be explained by various hypotheses which may require confirmation via pharmaco-kinetic studies, the fact that mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) significantly increased following supplementation, showed the positive effect of the supplement on the haematological parameters.

There was no significant difference between the median white cell count and median differential count of the patients before and after supplementation (table 6.1). The baseline total T-lymphocyte count [illegible]-lymphocyte count at the end of supplementation did not show significant differences (table 6.1). Lymphopaenia and neutropenia are common in HIV-positive/AIDS, especially in Africa (Wilson *et al.*, 2000). The present study did not observe this phenomenon.

The individual patients studied did not show thrombocytopenia, as the platelet counts were within the reference range (table 6.1). A high incidence of thrombocytopenia has been reported in some HIV-positive patients. A prevalence of thrombocytopenia of 5-12% has been reported in AIDS-Related Complex (ARC) patients compared with a prevalence of 30% in patients with AIDS (McPherdran, 1999). The mechanism of thrombocytopenia in HIV infection appears to be due to the presence of an autoantibody acting against a platelet membrane protein. This protein appears to be approximately 25 kilodaltons in size and is present on normal platelet. It may resemble an HIV precursor protein, but the exact mechanisms for platelet destruction remain unclear (McPherdran, 1999). For the patients in the current study, the non-detection of thrombocytopenia in the current study, even at baseline could be due to small sample size. Could an internal factor have been present that provided protection to the platelets against the destruction of platelets by the HIV? This demands further investigation

Different types of infections and clinical conditions have been reported in HIV-positive/AIDS patients (Maartens, 2001). The type and severity of the infection depend on the nature of the immune defect. Secondly, the degree of immune suppression is important as HIV infection causes a progressive immune suppression in all patients who are not treated with antiretroviral therapy. As immunity decreases, patients become susceptible to a wider spectrum of infections (Maartens, 2001). In addition, organisms that are prevalent in the patient's environment will be responsible for a large proportion of the infections. This is perhaps the reason why HIV-associated infections in sub-Saharan Africa differ markedly in their incidence from those in industrialised countries. These infections range from bacterial to fungal, parasitic and viral. The quality of life and lifespan of HIV-infected persons is affected by these various infections. In this study, a few opportunistic or HIV-related conditions/infections were identified and they are discussed below.

Few cases of HIV-related optha ems have been reported in HIV-positive/AIDS patients (Cunningham, 2001). The patients examined in the current study reported a very low infection rate (2.9%), with no jaundice or eye discharge (table 6.5). It is worth noting that even the very low eye infection rate documented did not progress as the study proceeded.

In this study, 28.6% of the patient population had an ear infection (table 6.5). Observation revealed that, the ear infection decreased to 17.2% (table 6.5) following supplementation. The patients were treated on recognition of the infection, therefore it makes it difficult for us to say with certainty whether the supplement had a positive effect on the clinical conditions. Nonetheless, the supplement probably aided in the healing or recovery process.

Studies (Maenza *et al.*, 1997; Tumbarello *et al.*, 1996; Sangeorzan *et al.*, 1994) have demonstrated the prevalence of oral infection due to Candida species in patients with HIV/AIDS. Greenspan (1994) reported that oral candidiasis is one of the most common oral infections in HIV patients. The most frequent species producing oral infection is *Candida albicans*, but other species are occasionally found which infect the oral mucosa (Greenspan, 1994). Both oral (22.9%) and throat (8.6%) candidiasis (table 6.5) were diagnosed among the studied patients. Candidiasis did not decrease during supplementation and treatment. The reason for this is not known, but drug resistance may be responsible. Fluconazole resistance in *Candida albicans* has been reported frequently in HIV/AIDS patients, especially those with $CD4^+$T-cell counts of less than 200 cells/mm^3 (Maenza *et al.*, 1997). Furthermore, a recent study indicated that dysfunction of $CD4^+$T-cells and $CD8^+$T-cells may be associated with inadequate defence against oral and oropharyngeal candidiasis in patients with HIV/AIDS (Douglas, 2003). As noted in this study, the $CD4^+$T-cell count decreased significantly, indicating a dysfunction in $CD4^+$T-cells. This could be another reason for the non-improvement in candidiasis following supplementation, but then it cannot be confirmed whether this mechanism (association between low $CD4^+$T-cell count and candidiasis) contributed to the clinical picture in regard to candidiasis. This needs further investigation.

In sub-Saharan Africa, *Mycobacterium tuberculosis* (TB) is the most frequent and in many instances the first opportunistic infection to occur in HIV-infected patients (Lucas *et al.*, 1993; Mortens & Low-Beer, 1996). of TB in HIV-infected patients has a poor prognosis and a high mortality rate (Nunn *et al*., 1992). HIV/AIDS patients also have a significantly higher risk of contracting TB infection owing to the underlying immune deficiency (Wilkinson & Moore, 1996). TB infection is a public health problem in South Africa, especially among the lower socio-economic group of society (Wilson *et al*., 2000). The prevalence of TB in this study was not high, occurring in 17.1% of the patient population (table 6.5). The clinical conditions improved with treatment and supplementation. From observation, TB does not seem to be a major infection in this group of patients. However, since the diagnosis of TB in these patients was based mainly on clinical features/symptoms rather than on radiological and microbiological methods, the prevalence of TB as reported here is subjective and the results need to be interpreted with caution. It is also worthy noting that viral load increases with TB infection, and the fact that the viral load in this study decreased significantly in the presence of TB infection, demonstrates the positive effect of the supplement and indicates the importance of supplementation in HIV-infected persons, especially those infected with of HIV and TB.

Swelling or enlargement of glands, especially near the neck, was observed in HIV-positive/AIDS patients (Maartens, 2001), but the severity and frequency of the swellings depended on the immune responses and the stage of HIV infection. In this study, clinical observation showed that the prevalence of swelling was higher around the jugular and it did not show improvement with supplementation (table 6.5).

Pathological abnormalities in the central nervous system have been reported in 7-90% of patients dying of AIDS (Levy *et al*., 1985; de la Monte *et al*., 1987). Cognitive dysfunction is said to be common as well. Immune deficiency may result in opportunistic infections of the central nervous system (CNS), causing specific neurological dysfunction (Navia & Price, 1987). The patients examined showed a normal state of consciousness (91.4%) and all demonstrated a normal sensory system. Ten (28.6) of the patients examined, (table 6.5) had neuropathy at baseline and showed no consistent sign of improvement with supplementation. The technique employed in the diagnosis of neurological problems was limited, thus certain abnormalities that could have been identified may have been left undiagnosed.

Malnutrition and body weight loss is ented in HIV infection (Niyongabo *et al.*, 1999; Hogan *et al.*, 2003). Malnutrition associated with HIV/AIDS is known to be the result of several processes, but the degree to which nutrition therapy can positively alter the course of HIV disease among HIV-positive/AIDS patients in Africa is largely unknown (Piwoz & Preble, 2000). Malnutrition in HIV/AIDS patients includes symptoms such as weight loss, loss of muscle and subcutaneous fat, vitamin and mineral deficiencies, reduced immune competence and increased susceptibility to infection. Conditions that could lead to malnutrition include lack of appetite, poor nutrient intake, limited food availability, chronic infection, malabsorption, metabolic disturbances, fever, nausea, vomiting and diarrhoea, depression and the side-effects of drugs (Sodeinde *et al.*, 1997; Taniguchi *et al.*, 1999). The medical history and medical examination of patients in the current study showed that 57.1% of the patients reported weight loss/ malnutrition (table 6.5). This condition improved with supplementation, falling to 34.5% (table 6.5). It is opined that the supplement partly contributed to this observed improvement in malnutrition. Another factor that could have contributed to the improvement of malnutrition is the fact that patients reported good appetite. Clinically, nausea and vomiting were not noted. This perhaps improved the nutritional status of the patients as well.

Gastrointestinal infections include parasitic, bacterial and viral which contribute to diarrhoea, especially in AIDS patients. Gastrointestinal pathogens include opportunistic organisms that cause severe intermittent gastrointestinal disease, and non-opportunistic organisms that usually cause acute treatable diarrhoeal illness (Smith *et al.*, 1992). Clinical assessment of the patients in the present study indicated that diarrhoea (8.6% at baseline) was not a prominent illness among the patients (table 6.5). This may be related to the adequate care provided by the home-based care. The patients were taught about personal hygiene and given health education.

Vaginal discharge resulting from either candidiasis or sexually transmitted infections was diagnosed in the patients during the period of study (table 6.5). Candidiasis seemed to predominate and the prevalence increased through out the study. This might be related to the chronic nature of the infection. It is important to subject such diagnosis to laboratory testing. This could lead to the particular causative agent being detected and isolated and where applicable, the antibiotic sensitivity pattern determined, and, ultimately appropriate treatment. Laboratory test is thus recommended in future studies.

Jeena (2001) reported that over 80-9 [illegible] e/AIDS patients have skin infections at some point during their lifetime. Local immunity of the skin is useful in determining the progression of HIV infection. With recurrent infections, there is impairment of the skin's immune system. Primary HIV infection produces a typical infectious mononucleosis-like rash (Jeena, 2001). Secondary mucotaneous involvement is common with viral, bacterial, fungal and other miscellaneous infections (Jeena, 2001). In this study, 28.6% (table 6.5) of the patients had skin rash at baseline but this decreased during the course of study. Ulcers remained stable through the course of study. The percentage of patients with skin infections, as indicated here, may be related to the impairment of the immune status (dysfunctional immune system).

6.7 Conclusion

This study showed that the supplement significantly reduced the viral load, suggesting that there may be some clinical benefit worthy of larger clinical trials. Since combinations of antiretroviral therapies are generally limited to privileged persons for economic, social, political and sometimes religious reasons, consideration of the potential of this supplement remains important for developing countries, particularly those in sub-Saharan Africa where a majority of the populace cannot afford antiretroviral therapy. It could have an appreciable benefit, perhaps similar to the effect of vitamin A supplementation on childhood mortality in developing countries.

Although, an ideal nutritional intervention strategy has not been devised, efforts to prevent nutritional complications and improve immune status and clinical conditions should include nutritional supplementation. This study showed that sterol/sterolin and vitamin/mineral supplementation of HIV-infected persons may have protective effects on the progression of HIV disease as indicated by the significant decrease in the viral load. The reduction in the viral load is very important since the median time is known to increase with a reduction in HIV viral load. The supplement also showed positive effects on a few of the haematological parameters and perhaps played a contributing role in improving some of the clinical conditions of the patients.

Nutritional management is basal to the care of all patients infected with HIV. Supplementation, unlike many other AIDS treatments has the potential in Africa to be an affordable and relatively easy-to-deli neasure. This study forms part of a comprehensive care system for the nutritional management of HIV-positive/AIDS patients. The supplement was well tolerated, hence no patient complained of any side-effects throughout the period of study. Observation showed that some of the patients improved physically and appeared healthier by the end of supplementation than before supplementation. Therefore, the benefit of the supplement may also extend to improved physical appearance and general well-being.

This study therefore, suggests that supplementing for longer periods (at least six months) could prove the effectiveness of the supplement in the clinical and nutritional management of HIV-positive/AIDS patients. Many vital questions, however, remain to be answered for one to understand fully the mechanism of action of the supplement and how best to utilize this new but potent therapy.

6.8 Limitation of the Study

This study was limited by small sample size, short duration and lack of a control group.

6.9 References

Allard JP, Aghdassi E, Chau J, Tam C, Kovacs CM, Salit IE *et al*. (1998). Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. **AIDS 12**: 1653-1659.

Altfeld M & Rosenberg ES (2000). The role of $CD4^+$T-helper cells in the cytotoxic T-lymphocyte response to HIV-1. **Curr Opin Immunol 12**: 375.

Bijlsma M (2001). Nutritional care and support for people with HIV/AIDS: review of initiatives and recommendations for developing national programmes in sub-Saharan Africa. Working paper for WHO: 1-6.

Bogden JD, Baker H, Frank O (1990). Micronutrient status and the human immunodeficiency virus (HIV) infection. **Ann New York Acad Sci 587**: 189-195.

Bouic PJD, Clark A, Brittle W, Lam[illegible]tone M & Liebenberg RW (2001). Plant sterol/sterolin supplement use in a cohort study of South African HIV patients. **S A M J 91** (10): 83-84.

Cunningham ET (2001). Unexplained vision loss in AIDS patients calls for florescein angiography. **Am J Ophthamol 132**: 727-733.

Chemiron International Ltd (1991). Documentation: 20-26

Clark SJ & Shaw GM (1993). The acute retroviral syndrome and the pathogenesis of HIV-1 infection. **Seminars in Immunology 5**: 149-155.

Coodley GO, Coodley MK, Lusk R (1996). Beta-carotene in HIV infection: An extended evaluation. **AIDS 10**: 967-973.

Coodley GO, Nelson HD, Loveless MO (1993). B-carotene in HIV infection. **J Acquir Immune Defic Syndr 6**: 272-276.

Coombs R, Welles SL, Hooper C (1996). Association of plasma human immunodeficiency virus type 1 RNA levels with risk of clinical progression in patients with advanced infection. **J Infect Dis 174**: 704-712.

Coutsoudis A, Bobat RA, Coovadia HM (1995). The effects of vitamin A supplementation on the morbidity of children born to HIV-infected women. **Am J Public Health 85**: 1076-1081.

Coutsoudis A, Moodley D, Pillay K (1997). Effect of vitamin A supplementation on viral load in HIV-1 infected pregnant women. **JAIDS 15**: 86-87.

Coutsoudis A, Pillay K, Spooner E (1999). Randomised trial testing the effect of vitamin A supplementation on pregnancy outcomes and mother-to-child HIV-1 transmission in Durban. South African Vitamin A Study Group. **AIDS 13**: 1517-1524.

Hansjee N, Kaufmann GR, Strub C, Weber R, Battegay M, Erb P (2004). Persistent apoptosis in HIV-1-infected individuals receiving potent antiretroviral therapy is associated with poor recovery of CD4T-lymphocytes. **J A I D S 36** (2): 671-677.

Harakeh S, Jariwalla RI, Pauling L (1990). Suppression of human immunodeficiency virus replication by ascorbate in chronically and acutely infected cells. **Proc Natl Acad Sci USA 87**: 7245-7249

Hogan JS, Weiss WP & Smith KL (2003). Role of vitamin E and selenium in host defence against mastitis. **J Dairy Sci 76**: 2795-2803

Itam IH (2003). The Effect of Chemiron on Haematological Parameters & Ferritin levels in Pregnant Nigerian Women in Calabar. **Mary Slessor J Med 3** (2): 17-24.

Jeena PM (2001): Common infections in South African children infected with HIV. **South Afri Med Assoc CME 19** (6): 352-358

Kanter AS, Cooper DC, Steinberg MH (1999). Supplemental vitamin B and progression to AIDS and death in black South African patients infected with HIV. Letter to the editor. **J A I D S 21**: 252-257.

Kennedy CM, Coutsoudis A, Kuhn L (2000). Randomized controlled trial assessing the effect of vitamin A supplementation on maternal morbidity during pregnancy and postpartum among HIV-infected women. **JAIDS 24**: 37-44.

Lamprecht J, Bouic PJD, Freestone & Austin M (1998). A prospective, controlled study to evaluate the effect of an essential sterol and sterolin formulation as a putative modulator in FIV (Feline immunodeficiency virus) infected laboratory cats. Presentation at the 12th World AIDS Conference, Geneva, June 18-July 3: 4-8.

Levy RM, Bredcson DF, Rosenblum ML (1985). Neurological manifestations of the acquired immunodeficiency syndrome (AIDS): experience at UCSF and review of the literature. **Neurosurg 62**: 475-495.

Lucas SB, Hounnou A, Peacock C (1993). The mortality and pathology of HIV infection of HIV infection in a West African city. **AIDS 7**: 1569.

Macallan DC (1999). Wasting in HIV infection and AIDS. **J Nutr 129**: 238S-242S.

Maartens G (2001). Infection in the HIV-infected patient. **South Afri Med Assoc CME 19** (6): 349-350.

Maenza JR, Merz WG, Romagnoli MJ, Keruly JC, Moore RD, Gallant JE(1997). Infection due to fluconazole-resistant candida in patients with AIDS: prevalence and microbiology. **Clin Infect Diseases 24**: 28-34.

Martin D (2000). Appropriate Laboratory Monitoring of HIV. **S A M J 90** (1):33-35.

McPherdran P (1999). Haematologic Complications of HIV. **Best Pract of Med 1**: 1-4.

Mortens TE & Low-Beer D (1996). HIV and AIDS: Where is the epidemic going? **Bull WHO 74**: 121.

Mellors JW, Rinaldo CR, Gupta Jr. P, White RM, Todd JA, Kingsley LA (1996). Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. **Science 272**: 1167-1170.

Miller TL & Gorbach SL (1999). Nutritional Aspects of HIV infection. London: Arnold Publishers: 199-211.

Moore RD (2000). Anaemia and human immunodeficiency virus disease in the era of highly active antiretroviral therapy. **Semin Haematol 37** (4) Suppl 6): 18-23.

Muslimatun S, Schmidt MK, Schultink W (2001). Weekly supplementation with iron and vitamin A during pregnancy increases haemoglobin concentration in Indonesian pregnant women. **J Nutr 13** (1): 85-90.

Navia BA, Price RW (1987). The acquired immunodeficiency syndrome dementia complex as the presenting or sole manifestation of human immunodeficiency virus infection. **Arch Neurol 44**: 65-69.

Niyongabo T, Henzel D, Idi M (1999). Tuberculosis, human immunodeficiency virus infection and malnutrition in Burundi. **Nutrition 14**: 289-293.

Nunn P, Brindle R, Carpenter L (1992). Cohort study of human immunodeficiency syndrome of patients with tuberculosis in Nairobi, Kenya. Analysis of early (6-month) mortality. **Am Rev Respir Dis 146**: 849.

O'Brien WA, Hartigan PM, Martin D (1996). Changes in plasma HIV-RNA and CD4 lymphocyte counts and the risk of progression to AIDS. Veteran Affairs Cooperative Study Group on AIDS. **N Engl J Med 334**: 426-431.

Oguntibeju OO, Van Schalkwyk FE, Van Den Heever WMJ & Veldman FJ (2003). Importance of Vitamin and Mineral Supplementation in HIV/AIDS Patients to improve their Nutritional and Immunological Status. **Pak J Med Sci 19** (3): 217-219.

Piwoz EG & Preble EA (2000). HIV/AIDS and nutrition: a review of the literature and recommendations for nutritional care and support in sub-Saharan Africa. A Report: 1-8.

Romeu MA, Mestre M & Gonzalez L (1992). Lymphocyte immunophenotyping by flow cytometry in normal adults: comparison of fresh whole blood lysis technique, Ficoll-Paque separation and cytopreservation. **J Immunol Methods 154**: 7-10.

Rowland-Jones S & Pinheiro S (2001). New insights into Host Factors in HIV-1 Pathogenesis: Minireview. **Cell 104**: 473-476.

SAHIVCS (2001). Course in HIV/AIDS Management 5:1-22.
Sangeorzan JA, Bradley SF, He X, Zarins LT, Ridenour GL, Tiballi RN *et al.* (1994). Epidemiology of oral candidiasis in HIV-infected patients: colonisation, infection, treatment and emergence of fluconazole resistance. **Am J Med 97**: 339-346.

SAS (1990). SAS procedures guide, version: 3rd ed, Cary NC: SAS Institute Inc: 705-991.

Semba RD & Tang AM (1999). Micronutrients and the pathogenesis of human immunodeficiency virus infection. **Br J Nutr, 81**: 181-189.

Singhai N & Austin J (2002). A clinical review of micronutrients in HIV infection. http://www.iapac.org/Text/ispring200201.htm. (Accessed on 18 June 2003).

Smith DS, Quinn TC, Strober W (1992) Gastrointestinal Infections in AIDS. **Ann Intern Med 116** (1): 63-77.

Sodeinde O, Adeyemo AA, Gbadegesin RA (1997). Persistent diarrhoea in Nigerian children aged less than five years: A hospital-based study. **J Diarhoeal Dis Res 15**: 155-160.

Taniguchi K, Rikimaru T, Yartley TE (1999). Immunological background in children with persistent diarrhoea in Ghana. **Pediatr Int 41**: 162-167.

Tumbarello M, Caldarola G, Tacconelli E, Morace G, Posteraro B, Cauda R et al. (1996): Analysis of the risk factors associated with the emergence of azole resistant oral candiasis in the course of HIV infection. **J Antimicrobial Chemotherapy 38**: 691-699.

UNAIDS (2002). HIV/AIDS Update, Fact Sheet: 1-12.

Van Staden AM, Barnard HC, Nel M, Attwood EM, Oosthuizen GM, Vander Ryst E *et al.* (1998). Nutritional status of HIV-1 seropositive patients in the Free State Province of South Africa-laboratory parameters. **Centr African J Med 44** (10): 246-250.

WHO (1993). WHO global programmes on AIDS. Proposed World Health Organization staging system for HIV infection and disease. **AIDS 7**: 868-869.

Wilkinson D & Moore DAJ (1996): HIV-related tuberculosis in South Africa-clinical features and outcome. **S Afri Med J 86**: 64-67.

Willumsen J (2003). The role of nutrition interventions in the prevention of HIV infection and progression of HIV/AIDS: Working paper for World Health Organization: 1-8.

Wilson D, Maartens G, Grimwood A & Wood R (2000). Clinical Guidelines for HIV Infection, South Africa: 2-8.

Wong GHW, McHugh T, Weber R, Goeddel DV (1991). Tumour necrosis factor alpha selectively sensitizes human immunodeficiency virus infected cells to heat and radiation. **Proc Natl Acad Sci USA 88**: 4372-4376.

Yang OO (1998). CD8T-cells in HIV Infection: Mechanisms of Immunity. **Hospital Practice 11**: 1-20:http://www.hosppract.com/issues/1998/11/yang.htm (Accessed on April, 2004)

7 General Summary

Evidence from literature indicates that, although nutritional factors are not likely to be the most significant aetiological determinants in HIV infection, carefully chosen nutritional support or supplementation in terms of quality and safety can improve nutritional status and immune function. It has the potential to retard viral expression and disease progression, thereby improving the general well-being of people living with HIV/AIDS.

Furthermore, nutrient supplementation in HIV-infected individuals is likely to be more important among under-privileged populations, but unfortunately very few studies have been reported in developing countries. The HIV prevalence in South Africa and the rest of Africa is becoming critical as the epidemic shifts towards the more socially and economically disadvantaged populations, with a concomitant reduction in house income earnings and food production. Since strong relationships exist between food production/availability and nutritional status and between nutritional and immune status, these implications adversely affect both the nutritional and immune status especially of people living with HIV/AIDS. Therefore, a majority of people living with HIV/AIDS may possibly be at risk of malnutrition and micronutrient deficiencies due to inadequate intake or because of an increased metabolic rate resulting from the infection.

The aim of this study was to examine the influence of a nutritional supplement (Africa's Solution) on the immune status of HIV-positive/AIDS patients who are economically disadvantaged. The results and conclusions of this are envisaged to have a broader impact in the industry as well as to the patients and have academic benefits.

The study analysed the baseline dietary intake of 35 HIV-positive/AIDS patients. A food frequency questionnaire was used to assess both the macronutrient and micronutrient intakes of the patients at baseline. It is known that nutrient the requirements for HIV-positive/AIDS patients are higher than for individuals who are HIV negative, because of the catabolic activity of HIV infection. However, the exact nutritional requirements for HIV-positive/AIDS patients are not known, hence the recommended daily allowances/adequate intake (RDA/AI) was used as a standard reference so as to allow for comparison of the results of the study with those of previous studies. In this study the patients demonstrated energy and dietary intake of major macronutrients higher than the RDA/AI and higher ($P<0.05$) in males than in females. The results also indicated that the mineral and trace element intakes exceeded the RDA/AI, except for iodine and selenium. The majority of patients reported adequate intake of most vitamins with the exception of folate and vitamin D. It is envisaged that the high dietary intake of major macronutrients and micronutrients will help in maintaining the nutritional status and in curtailing wasting in the patients.

The influence of the supplement on anthropometric profiles and their association with the $CD4^{+}$T-cell count and viral load were determined. The anthropometric profiles and the viral load of the patients were determined at baseline (n= 35) and by the end of the study (n=28) while the CD4+T-cell count was performed monthly during the period of the study using standard procedures. Results showed that there was no significant difference ($P>0.05$) in the fat percentage and body weight before and after nutrient supplementation; however, the fat percentage differed significantly ($P<0.05$) according to gender. In general, the body mass index (BMI) and the lean body mass (LBM) produced a trend towards an improvement. There was a positive correlation between BMI and fat percentage. The $CD4^{+}$T-cell count showed no correlation with the anthropometric profiles, while the viral load showed a negative correlation with the LBM, the fat percentage, and the BMI, but indicated a positive correlation with the WHR.

The influence of the supplement on the immune status, haematological parameters and clinical conditions of the patients were also tested. The results showed that the viral load decreased significantly ($P<0.002$) with time following supplementation. The mean cell volume (MCV) and the mean cell haemoglobin concentration (MCHC) increased significantly ($P<0.002$, $P<0.0002$) respectively, reflecting the positive effect of the supplement on a few of the haematological parameters. The supplement demonstrated no effect on the $CD4^{+}$T-cell count and the $CD4^{+}$T-cell count decreased significantly ($P<0.05$) with HIV disease progression. The non-positive effect of the supplement on the $CD4^{+}$T-cell count may be related to the already low $CD4^{+}$T-cell counts before supplementation (lower than 200 cells/mm^3 in majority of the patients), short duration, inter-assay variation, changes due to inter-current illness, impaired production, redistribution within the intravascular spaces, drug-nutrient and food-nutrient interactions. The supplement showed unconfirmed but observable positive effects on the general well-being (clinical conditions) of the patients.

In general, the study population demonstrated an adequate intake of energy, major macronutrients and micronutrients. The BMI and LBM produced a trend towards improvement. The viral load decreased significantly, while the MCV and MCHC increased significantly with supplementation. The physical appearance and the general well-being of the patients improved following supplementation. However, the supplement did not indicate a positive effect on the $CD4^{+}$T-cell counts, possibly due to the short duration of supplementation, stage of HIV infection (late stage), low level of immune status, inter-assay variation and degree of bioavailability of supplement. The reduction in the viral load is very important since median survival time is known to increase with reduction in the HIV viral load. Because of certain limitations (small sample size, short duration, late stage of the infection and inter-assay variation), further study is necessary to confirm the influence of the supplement.

7.1 Recommendations

It is recommended that:

- Future study should recruit HIV-positive patients with a higher $CD4^{+}$T-cell count as inclusion criterion because of increased chances of patients' drop out resulting from low $CD4^{+}$T-cell count.
- There is need to assess the dietary intakes at both baseline and end of study as this would give a better assessment of the nutritional status of the patients as the infection progresses.
- Controls should be included in subsequent studies for the purpose of comparison and to be able to monitor the effect of the supplement more reliably and effectively.
- There is the need for a larger prospective population-based study of both HIV-positive patients and negative (control) individuals.
- The duration of supplementation should be extended to six months or one year for possible better effects.
- In subsequent studies, laboratory analysis of blood vitamins and minerals should be carried out before, during and after supplementation. This would assist in monitoring the possible effects of the supplement.
- More research is needed in order to find out or define more clearly the health-risk posed by HIV infection and to establish meaningful criteria for the formulation of interventions with regards to the health and nutrition of HIV-infected persons.

- Consumption of alcohol should also be monitored in HIV-positive/AIDS patients since consumption of alcohol or related substance is known to have adverse effects on nutritional status.

Central University of Technology, Free State

APPENDIX A

SOCIO-DEMOGRAPHIC QUESTIONNAIRE
(All information in this questionnaire is confidential).

Name:__________________

Respondent number: 1-3

Interviewer: __________________ 4-5

D D M M Y Y Y Y

Birth Date: 6-13

Interview Date: 14-21

Age (years) if Birth Date unknown:____________________ 22-23

Address: ____

Tel No (H):___________________ (W):___________________

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

____

Encircle the appropriate answer:

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**

Do you smoke at all? 31

1. Yes
2. No

If yes, how many cigarettes per day? 32-33

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**

Marital status of respondent: 40

1. Unmarried
2. Married
3. Divorced
4. Separated
5. Widowed
6. Living Together
7. Traditional Marriage
8. Other, specify_______________

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

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

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

Who is the head of this household? 44

1. Self
2. Husband
3. Child/ren
4. Parent
5. Grandparent
6. Friend
7. Other, specify _______________

Type of dwelling: 45

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

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

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 ________________

What type of toilet does this household have? 49

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

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

Do you use a cast iron pot for cooking? 51

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

Does the home have a **working**:

Refrigerator and/or freezer 52

1. Yes
2. No

Stove (Gas, Coal or electric) or Hot Plate 53

1. Yes
2. 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

How many people contribute to the total income? ___________ 57-58

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

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

1. Yes
2. No

If no, is it more or less? 61

1. More
2. Less

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 R 400

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APPENDIX B: Anthropometry

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

Bio-impedance:

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 C

QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

Name:___________________________ 1-3

Respondent number:

Interviewer:___________________________ 4-5

QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

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 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):

- 1.More than three meals with eating between meals 27
- 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.Never	2.> once/week	3.Weekly	4.Monthly	5.> once a month		33

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			
			Per day	Per week	Per month	Seldom Never
Maize-meal porridge	Stiff (pap)					
Maize-meal porridge	Soft (slappap)					
Maize-meal porridge	Crumbly (phutu)					
Sour porridge	Specify ratio Mabella/Maize					
Mabella porridge	Stiff, coarse, fine					
Mabella porridge	Soft, coarse, fine					
Oats porridge	Brand name:					
Breakfast cereals	Puffed Wheat, plain					
	Corn Flakes, plain					
	Weet Bix					

	Puffed Rice, sweet					
	Specify types usually eaten _____________________ _____________________ Brand names of cereals available at home now:___________________					
Milk on porridge or cereal: Circle type usually used	None					
	Whole/fresh					
	Sour					
	2% fat					
	Fat free/skimmed					
	Milk blend					
	Soy milk					
	Condensed (whole,sweet)					
	Condensed (skim, sweet)					
	Evaporated whole					
	Evaporated low fat					
	Non-dairy creamer					
Is sugar added to porridge or cereal? (Tick box)	None White Brown Syrup Honey Sweetener: type___________					
Is fat added to porridge or cereal? (Tick box)	None Animal fat (butter) Hard margarine Soft margarine Oil Peanut Butter					

Samp/Maize rice **Samp and beans** **Samp and peanuts**	Bought Self ground Specify ratio (1:1) Specify ratio					
Rice: specify brands names:	White Brown Sorghum rice					
Stamped wheat						
Pastas	Macaroni Spaghetti Spaghetti in tomato sauce Other:					

HOW MANY TIMES A WEEK DO YOU EAT PORRIDGE OR BREAKFAST CEREAL AT ANY TIME OF THE DAY (NOT ONLY BREAKFAST)?____________

FOOD	DESCRIP-TION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom/ Never		
Bread/Bread rolls **Bread slices: thin** **Medium, thick**	White						**3210**	
	Brown						**3211**	
	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**	Cheese, tomato & onion __________						**3353**	

sausage) Hamburgers (specify meat)	____________ __ ____________ ____________ ____________ ____________ ____________ ____________ ____________ ____________ __________							
Are any 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						**2760 2725 2723 2722**	

	Cheddar Other:____ __________ __ __________ __________ __							
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	Boiled: with skin without skin						2926 2963	
Do you eat the chicken with the skin?	Fried: in batter/crumbs Fried, but not coated						3018 2925	
Yes No	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:						300	

	with fat without fat						6 290 9	
	Mince with tomato and onion						298 7	
Red meat: Mutton	Fried/grilled: with fat without fat						292 7 293 4	
	Stewed/boiled: with fat without fat						304 0 291 6	
Red meat: Pork	Fried/grilled: with fat without fat						293 0 297 7	
	Stewed/boiled: with fat without fat						304 6 304 5	
Red meat: Goat	Fried/grilled: with fat without fat						428 1	
	Stewed/boiled: plain with veg						428 1 428 2	

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 vegetables 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**	

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						**2867**	
	Scrambled in: oil						**2889**	
	butter						**2886**	
	margarine						**2887**	
	Fried in: oil						**2869**	
	butter						**2868**	
	margarine						**2877**	
	bacon fat						**2870**	
	Curried						**2902**	

HOW MANY TIMES A WEEK DO YOU EAT MEAT __________,

BEANS __________,

CHICKEN __________,

FISH __________ **AND**

EGGS __________**?**

FOOD	**DESCRIPTION**	**AMOUNT USUALLY EATEN**	**TI**	
			Per day	**Per weel**
Cabbage	Boiled, nothing added			
	Boiled with potato and onion and fat			
	Fried, in margarine (nothing added)			
	Fried, in oil (nothing added)			
	Boiled, then fried with potato, onion			
	Other:			
Spinach/morogo/imfino/other green leafy vegetables: List names	Boiled, nothing added			
	Boiled fat added (margarine)			
	Boiled with onion/tomato and fat			
	-onion &potato (margarine)			
	- onion, tomato & potato			
	- with peanuts			
	Other:			

Tomato and onion 'gravy'/relish/chow	without fat			
	Canned			
Pumpkin Specify type:	Cooked in fat & sugar			
	Boiled, little sugar and fat			
	Boiled			
________________	Other:			
Carrots	Boiled, sugar & fat			
	Boiled, nothing added			
	Boiled, potato, onion, no fat			
	Boiled, potato, onion, margarine			
	Boiled, with sugar			
	With potato/onion			
	Raw, salad (orange juice)			
	Chakalaka			
	Other:			
Mealies/Sweet corn	On cob			
	Off cob -creamed sweet corn Off cob whole kernel			
Beetroot	Cooked Salad (bought or home-made)			

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT /DAY
			Per day	Per week	Per month	Seldom / Never		
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 -						**3876**	

	whole milk, margarine						
	Roasted in beef fat					**3878**	
	French fries/potato chips (oil)					**3740**	
	Salad (mayonnaise 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 Green, frozen with sugar With sugar and butter Tinned peas					**4146** **3720** **3859** **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	Sauteed in					**3730**	

	sun oil Sauteed in margarine						**3844**	
Salad vegetable s	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**	
Cauliflow er	Boiled ______						**3716**	
Other vegetable s; 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	T 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, unsweetened						**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) Peach (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	TIMES TAKEN	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	Fresh/long life whole						**2718**	
What type of milk do you put in tea and/or coffee?	Fresh/long life 2% Goat						**2772** **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 (skim)						2744	
	Evaporated milk (whole)						2715	
	Evaporated milk (low-fat)						2827	
	None							
Milk as such: What type of milk do you drink as such?	Fresh/long life/whole Fresh/long life/2% Fresh/longlife/fat free (skimmed) Goat						2718 2772 2775 2738	
	Sour / Maas						2787	
	Buttermilk						2713	

BEVERAGES	**DESCRIPTION**	**AMOUNT USUALLY TAKEN**	**TIMES TAKEN**				**CODE**	**AMOUN /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** **400**	

							4	
Sweets	Toffees Hard boiled Fudge, caramels (specify)						**399 1 398 6 399 1**	
Biscuits/cookies	Specify type Home made plain Shortbread, butter Commercial, plain Commercial with filling						**323 3 329 6 321 6 321 7**	
Cakes & tarts	Chocolate, plain						**341 9**	
Pancakes/ crumpets							**334 4**	
Koeksisters							**323 1**	
Savouries	Sausage rolls Samoosas - vegetable Samoosa - mutton Biscuits e.g. bacon kips Other:						**293 9 341 4 335 5 333 1**	
Pudding: jelly							**398 3**	
Baked pudding	Plain batter						**342 9**	
Instant pudding	Skim milk Whole milk						**331 4 326 6**	
Ice cream	Commercial regular Commercial rich Soft serve Sorbet Ice lollies Chocolate coated individual ice creams (e.g. Magnum)						**348 3 351 9 351 8 349 1**	

							3982	
Custard	Home made, whole milk Ultramel						2716 2716	
Cream	Fresh						3520/ 3480	
Other puddings (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/chick							402	

en stock							9	
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

Central University of Technology, Free State

APPENDIX D

PHYSICAL EXAMINATION

(All information in this questionnaire is confidential).

Name:__________________

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

Interviewer: _________________ [ ][ ] 4-5

	D	D	M	M	Y	Y	Y	Y	
Birth Date:									6-13
Interview Date:									14-21
Age (years) if Birth Date unknown:__________________									22-23

Address: ____

Tel No (H):__________________ (W):__________________

SYSTEM	YES	**NO**
1. **EYES**		
• JAUNDICE		
• DISCHARGE		
• INFECTION		
• PUPIL NORMAL		
2. **EARS**		
• PAIN		
• INFECTION		
• DISCHARGE		
• VERTIGO		
3. **NOSE**		
• PAIN		

• ULCER • SWELLING		
4. MOUTH AND LIPS • PAIN • ULCER • SWELLING • CRACKED LIPS • CANDIDAS		
5. THROAT • PAIN • ULCER • INFECTION • CANDIDAS		
6. NECK • SWELLING # SUBMENTAL # JUGULAR # POSTERIOR TRIANGLE OF NECK # OCCIPITAL		
7. BREASTS • **PAIN** • **LUMPS** **DISCHARGE**		

8. RESPIRATORY SYSTEM • COUGH • SHORTNESS OF BREATH/ DYSPNOEA • PAIN • HAEMOPTYSIS • TB • ABNORMAL BREATH SOUNDS		
9. CARDIOVASCULAR • SWELLING OF LEGS/ANKLES • OTHER EDEMA • SHORTNESS OF BREATH/DYSPNOEA • CYANOTIC • CLUB FINGERS		
10. BLOOD FORMING ORGANS • ENLARGEMENT OF SPLEEN • ` GLANDS IN NECK • `` AXILLA • GROINS		
11. DIGESTIVE • MASS LOSS/MALNARISH • EATING WELL • NORMAL BOWEL MOVEMENTS		

• STOOLS NORMAL • ABDOMINAL PAIN • NAUSEA/VOMITING • PAIN • LIVER ENLARGEMENT • SPLEEN ENLARGEMENT		
12. **GENITO-URINARY** • DYSURIA • DYSMENORRHOEA • VAGINAL DISCHARGE • URETHRAL DISCHARGE • STI • PENILE DISCHARGE • SWELLING OF SCROTUM		
13. **LOCOMOTOR** • PAIN • NEUROPATHY		
14. **SKIN** • RASHES • ULCERS • TUMORS		
15. **CENTRAL NERVOUS** • PARALYSIS • NORMAL STATE OF CONSCIOUSNESS		

Central University of Technology, Free State

• NORMAL SENSORY SYSTEM **16. VITAL SIGNS** • TEMP: • BLOOD PRESSURE • RESP • PULSE		
17. TREATMENT :		

APPENDIX E: Consent Form

HIV/AIDS Nutrition Project

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

A.	Patient information
Surname:	--
First name	--
Male: ---------------	**Female**: -----------------
Date of birth:	-----------------------------

B. Patient's Consent

Hereby I declare that I have been informed of the aims of this project (as printed at the back of this form) and that the following samples can be taken: 2 tubes of blood (purple stopper blood). I also understand that my information will be regarded as confidential, that my participation is voluntary and that I could withdraw at any time.

______________________ ______________________

Patient's signature Date

C. Information sheet

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. Africa's Solution was developed and this clinical trail is set up to investigate its efficiency on the immune system of HIV/AIDS patients with a suppressed immune system.

HIV/MIV JEK

Datum: -----------------

A. Pasient inligting

Van: ---

Volle Name ---

Manlik: --------------- **Vroulik:** -----------------

Geboorte datum: ------------------------------

B. Pasient Toestemming

Hiermee verklaar ek dat ek ingelig is oor die doelwitte van die projek (soos uiteengesit op die agterblad) en die volgende monsters kan van my geneem word: 2 buise bloed (pers prop buise). Ek verstaan ook dat my pasient inligting konfidensieel hanteer sal word en dat my deelname vrywillig is en ek teen enige tyd kan onttrek.

____________________ ____________________

Pasient handtekening Datum

C. Inligtingstuk

Verskeie vitamiene en minerale is krities vir die bevegtig van HIV infeksie, omdat hulle benodig word deur die immuunsisteem en die belangrikste organe om geinfekteerde patogene aan te val. Navorsers het aangedui dat gedurende die vroeë fase van HIV infeksie gewigstoename en instandhouding verkry kan word deur voeding. Data oor die voedingsstatus, dieët inname en /of supplement aanvulling in HIV geinfekteerde persone in ontwikkelende lande is redelik maklik beskikbaar. Hierdie inligting is egter nie so maklik verkrygbaar in Afrika nie, waar wanvoeding en ondervoeding redelik algemeen is. Daarom het dit nodig geraak om die rol van 'n supplement aanvulling in HIV positiewe pasiente te evalueer, veral in ontwikkelende lande. "Africa's Solution" is ontwikkel en 'n kliniese studie is saamgestel om die invloed van die supplement aanvulling op die immuunsisteem te ondersoek in HIV/MIV positiewe pasiente met 'n onderdrukte immuunsisteem.

HIV / AIDS ... OJECT

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

Hlahiso lesedi ya monkakarolo

Sefane: ....

Lebitso: ....

Monna: .... Mosadi: ....

Letsatsi la tswalo: ....

Tumallano ya monka karolo

Kaho ke paka hore ke tsibisitswe ka maikemisetso a projeke ena (kaha ho tlanyaditswe ka morao ha foromo ena) le hore ho ka etwa dipatlisiso tse latelang: ho nkuoa ha madi ka di dinnalete tse pedi (e le nywe ya sekwahelo se kgubedu, le enywe ka se perese). Kutlwisiso ya ka kehore ntho tsaka di tla amohelwa ka sephiring, le hore honka karolo haka ho bolela hore nka tlohela haro ho sebaka.

__________________ __________________

Tshaeno ya monka karolo Lestatsi