Evaluation of the effects of an instant soy and maize meal supplement on the vitamin A status of patients infected with the human immunodeficient virus

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Mini-dissertation submitted in the School for Physiology, Nutrition and Consumer Sciences of the Potchefstroomse Universiteit vir Christelike Hoër Onderwys (PU for Christian Higher Education) in partial fulfilment of the requirements of the degree Magister Scientiae (Nutrition)

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Potchefstroom
2001

Potchefstroomse Universiteit
vir Christelike Hoër Onderwys

ACKNOWLEDGEMENTS

I hope the content of this project will be useful for others who wish to undertake further work on both the benefits of soy and the role of vitamin A in HIV/AIDS. This project has been a combined effort of many people, without their support it would not have been completed. I therefore am obliged to acknowledge the following people.

My study leader, Professor C.S. Venter, who guided me throughout the process of conducting the research, analysis and report writing.

Professor J.C. Jerling for his patience and guidance is assisting me with the statististical analysis and interpretation of the results.

Dr. W. Oosthuizen, Sister C. Lessing and Ms. D. Loots for facilitating the handling, processing and analysis of the biochemical and clinical aspects of the study.

Captain M. Mooko, Ms. F. Labuschagne, Ms. W. Dercksen, Mrs K. Steyn, and Ms. R. van der Merwe, fellow post-graduate students who were also responsible for specific components of the project, and were always available to give support when required.

Mrs. A. Roux and Mrs. C. Wessels for their valuable contribution in the social support therapy sessions.

Ms. H. Vorster for assisting in the collection of dietary intakes and providing guidance in analyzing the data.

Mrs. S. Legoete and Ms. M. Maki, the field workers. Their contribution of interviewing the study subjects is one of the integral components of this research.

Mrs. M. Terblanche for editing the final dissertation, and Dr. W. Oldewage-Theron, the external examiner for this mini-dissertation.

The personnel of Ferdinand Postma Library, especially Ms. Louise Vos and other staff in the Interlibrary Loans Department, for their friendly, expert assistance at all times.

Mr. and Mrs. Oosthuizen of Rysmierbult farm for the support they provided to both the research team and the study subjects throughout the study.

SPP and Sportron International for the respective donations that they provided during the study.

The Department of Health, in Potchefstroom, Ventersdorp, Wedela, Fochville and Klerksdorp North for granting permission for the study to be conducted in their respective areas.

Tšepong HIV/AIDS support group, nursing sisters and HIV/AIDS counsellors in the clinics of the above mentioned health service areas, for their support and participation during recruitment of the subjects and at data collection sessions.

All the subjects who were eager and patient to participate in the project, especially as it is appreciated that the condition they are in required to be handled with very strict confidentiality.

Ms. H. Langenhoven and Mrs. E. De kock (Potchefstroom University, Department of Nutrition) for the endless support I received from them during the course of this study.

Nestlé South Africa and Switzerland, and the government of Lesotho for expressing an interest in this particular research topic and supporting me to undertake this Master of Science post-graduate course in nutrition.

The National Research Foundation for the financial support.

My husband Moses and daughter Tiisetso for all the love, support and patience they gave me during this study.

Above all I do thank God for being with me throughout my studies.

ABSTRACT

The development of a culture sensitive nutritional supplement for people infected with the human immunodeficiency virus (HIV) in developing countries needs to be investigated by experts in the area of nutrition and dietetics. Evidence from studies has shown that nutrients (micronutrients and phytochemicals) have the potency to delay progression of HIV into acquired immunodeficiency syndrome (AIDS). The soybean is a locally available culturally accepted food within the Southern Africa region with a relatively adequate nutrition profile, complementing the staple food maize meal excellently. The bean is also a source of vitamin A, one of the nutrients that have been implicated to play a role in maintenance of an integral immune system. Little is understood of the effect of soybeans in HIV/AIDS disease progression.

This placebo controlled trial was therefore implemented to assess the effect of an instant soy maize meal supplement (SMMS) in free living HIV-positive adults (n=16) in the North West Province in South Africa. The control group (n=12) took a placebo and a low-dose multivitamin tablet, while the intervention group took 300g daily of the SMMS and the multivitamin tablet. At baseline, midline (after three months), and end (after six months), assessments were performed on selected dietary nutrient intakes, anthropometric and biochemical variables and quality of life as measured by a validated questionnaire. In many cases the small numbers of subjects made it difficult to draw meaningful conclusions. At the end of the six months intervention the mean nutrient intakes of the SMMS group was increased by the supplement relative to that of the controls. The anthropometric measurements of both groups remained the same over six months. Markers for disease progression such as CD4 count and CD4 as percentage of total lymphocytes showed a decrease in the SMMS group ($p<0.01$ and $p=0.05$ respectively). Serum retinol levels were within the adequate range for both groups at baseline and end, though these levels tended to decline with a decrease in CD4 count. The SMMS group experienced a greater non-significant decrease in the mean serum retinol concentration at -1.2 μmol/L relative to –0.04μmol/L for the control group. The

decrease in serum retinol binding protein was greater in the SMMS group at –0.9mg/L ($p=0.02$) relative to the controls at –0.5mg/L ($p=0.34$). Quality of life improved in both groups ($p=0.0005$ in the control group). The SMMS group had started with higher scores than the control group, and the room for improvement was limited. Viral loads did not change significantly.

The SMMS did not show any beneficial effect on the progression rate of HIV in this small group of subjects. However, the effect of the intervention might have been masked by the multivitamin tablet, which was taken by both groups. There is therefore a need for further research on the effect of SMMS with a bigger sample size. In anticipation of the relatively high withdrawal rate in studies with HIV/AIDS patients, at least double the number of subjects needed for detecting significant effects should be entered into such a trial. It is further recommended that the SMMS be fortified with vitamin A in quantities two to three times the recommended dietary allowance.

OPSOMMING

Die ontwikkeling van 'n kultuursensitiewe voedingsupplement vir persone besmet met die menslike immuniteitsgebrekvirus (MIV) in ontwikkelende lande, moet deur kundiges op die gebied van voeding en dieetkunde ondersoek word. Bewyse van studies het getoon dat nutriënte (mikronutriënte en fitochemikalieë) oor die vermoë beskik om die vordering van MIV na verworwe immuniteitsgebreksindroom (VIGS) te vertraag. Die sojaboon is 'n plaaslikbeskikbare kultuuraanvaarbare voedsel in die Suidelike Afrikastreek, met 'n relatief toereikende nutriëntprofiel, wat die stapelvoedsel mieliemeel uitstekend aanvul. Die boon is ook 'n bron van vitamien A, een van die nutriënte wat 'n rol speel in die handhawing van 'n integrale immuunstelsel. Min is bekend aangaande die effek van sojabone in MIV/VIGS-siekteprogressie.

Hierdie plasebogekontroleerde ondersoek is daarom uitgevoer om die effek van 'n kitssoja-mieliemeelsupplement (SMMS) in vrylewende MIV-positiewe volwassenes (n=16) in die Noordwes-Provinsie in Suid-Afrika te ondersoek. Die kontrolegroep (n=12) het 'n plasebo en 'n lae-dosis multivitamientablet ontvang, terwyl die intervensiegroep daagliks 300g van die SMMS en die multivitamientablet ingeneem het. Met basislyn, middellyn (na drie maande) en aan die einde (na ses maande) is bepalings gedoen van geselekteerde nutriëntinnames, antropometriese en biochemiese veranderlikes en kwaliteit van lewe, soos gemeet met 'n geldige vraelys. Die klein getal proefpersone het in baie gevalle sinvolle gevolgtrekkings uit die resultate bemoeilik. Aan die einde van die ses maande lange ingreep was die gemiddelde nutriëntinnames van die SMMS-groep deur die supplement verhoog, relatief tot die kontroles. Die antropometriese veranderlikes van beide groepe het dieselfde gebly gedurende die ses maande. Merkers vir siekteprogressie, soos CD4-seltelling en CD4 as persentasie van totale limfosiete, het 'n verlaging in die SMMS-groep vertoon ($p<0.01$ en $p=0.05$, respektiewelik). Serumretinolvlakke was binne aanvaarbare grense vir beide groepe met basislyn en aan die einde, hoewel hierdie vlakke geneig het om te daal met 'n daling in CD4-telling. Die SMMS-groep het 'n groter nie-betekenisvolle verlaging in die gemiddelde serumretinolkonsentrasie ervaar naamlik –1.2 µmol/L relatief tot –0.04 µmol/L vir die kontrolegroep. Die verlaging in

serumretinolbindende proteïen was groter in die SMMS groep naamlik –0.9 mg/L (p=0.02) relatief tot die kontroles (-0.5mg/L, p=0.34). Lewenskwaliteit het in beide groepe verbeter (p=0.0005 in die kontrolegroep). Die SMMS-groep het aanvanklik 'n hoër telling as die kontrolegroep gehad, en die ruimte vir verbetering was beperk.

Die SMMS het nie enige voordelige effek op die progressiesnelheid van HIV in hierdie klein groep proefpersone gehad nie. Die effek van die intervensie kon egter verbloem gewees het deur die multivitamientablet wat deur beide groepe ingeneem is. Dus is dit nodig dat verdere navorsing gedoen moet word aangaande die effek van SMMS met 'n groter steekproef. In die lig van die relatief hoë uitvalsyfer in ondersoeke met MIV/VIGS-pasiënte, moet ten minste dubbeld die aantal proefpersone nodig om betekenisvolle effekte op te spoor, in so 'n studie aanvaar word. Dit word verder aanbeveel dat die SMMS met vitamien A verryk word in hoeveelhede twee tot drie keer die aanbevole dieettoelae.

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AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
ARI	AIDS related infections
AST	Aspartate aminotransferase
AZT	Zidovudine
B	Baseline
β	Beta
BFA	Behring fibrintimer
BMI	Body mass index
BMR	Basal metabolic rate
CDC	Centers for disease control
CI	Confidence interval
cm	Centimetre
CRP	C-reactive protein
^{0}C	Degrees centigrade
DL	Decilitre
DNA	Deoxyribonucleic acid
DRIs	Dietary reference intakes
E	End
EI	Energy intake
ELISA	Enzyme linked immunosorbent assay
FAO	Food and Agriculture Organisation
FNB	Food and nutrition board
g	Grams
HDL	High density lipoproteins
HDL-C	High density lipoproteins cholesterol
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
IEC	Information education and communication

IU	International units
IVACG	International Vitamin A Consultative Group
kg	Kilograms
kJ	Kilojoules
L	Litre
LD	Lactate dehydrogenase
LTD	Limited
ml	Millilitre
mg	Milligram/s
MI	Micronutrient initiative
mm	Millimetre
mm^3	Cubic millimeter
n	Number
nm	Nanometer
NFCS	National Food Composition Survey
NKC	Natural killer cells
µg	Microgram/s
µL	Microlitre
µmol	micromole
µgRE	Micrograms retinol equivalents
OR	Odds ratio
p	Level of significance
PDCAAS	Protein digestibility corrected amino acid score
PEM	Protein energy malnutrition
PTY	Proprietors
PU for CHE	Potchefstroom University for Christian Higher Education
QFFQ	Quantitative food frequency questionnaire
r	Pearson's correlation coefficient
RBP	Retinol binding protein
RDA	Recommended dietary allowances
RE	Retinol equivalents

CHAPTER 1

BACKGROUND TO THE PROBLEM: MOTIVATION FOR THE STUDY

1.1 Introduction

The acquired immunodeficiency syndrome (AIDS) was first described by the Centers for Disease Control (CDC) in 1981 (Mahan & Escott-stump, 2000). In 1983, researchers isolated the etiologic agent, a retrovirus which was named immunodeficiency virus (HIV). Since then 360, 000 cases of persons diagnosed with AIDS have been reported in the United States. In April 1995, the CDC reported that 53% of these cases were caused through male-to-male contact and 25% by intravenous drug use. Others affected include heterosexuals, haemophiliacs and other recipients of blood transfusions and infants born to mothers with AIDS (Mahan & Arlin, 2000). Globally, it is estimated that in 1999, 33.4 million people were infected by the virus, of whom two-thirds are in Sub- Saharan Africa. In October 1999, 4.1 million South Africans out of the total population of 42 million were HIV positive. It is estimated that in 2006 more people in South Africa will die from AIDS than from any other cause, and all other causes combined. It is therefore, clear that the HIV and AIDS epidemic is a world-wide and national emergency (Vorster, 2001).

1.2 Relationship between malnutrition and AIDS

Malnutrition is an important and complicated consequence of HIV infection. Protein-energy malnutrition (PEM) is a frequent complication of AIDS. Weight loss, body cell mass depletion, decreased skinfold thickness and midarm circumference, decreased iron-binding capacity, and hypoalbuminaemia are frequently reported (Mahan & Arlin, 2000).

AIDS wasting syndrome is the second most commonly reported AIDS-defining condition for adults, and the fourth for children younger than 13 years of age (CDC, 1996). Weight loss and wasting are considered multi-factorial, with major contributing factors being: lack of adequate intake, malabsorption, metabolic irregularities, uncontrolled opportunistic infection, and lack of physical activity. Decreased oral intake, identified as the most common cause of weight loss, can be the result of anorexia secondary to medications; depression; infection; symptoms, such as nausea, vomiting, diarrhea, dyspnoea, or fatigue; or neurologic disease (Mahan & Escott-Stump, 2000).

Low oral intake can also be attributable to disorders of the mouth and oesophagus, such as candidiasis or herpes simplex. Malabsorption, often suspected when there are loose stools, diarrhea, or vomiting, can be caused by the HIV infection; opportunistic infections, or developed intolerances to lactose, fat, and possibly gluten. At the same time, energy and protein needs may be increased by fevers and infection. Resting energy expenditure is elevated in asymptomatic HIV-infected persons and relates to viral load (Mulligan et al., 1997). Malnutrition may contribute to the frequency and severity of infection seen in AIDS by compromising immune functions (Chlebowski, 1985). Deficiencies of protein, energy, copper, zinc, selenium, iron, essential fatty acids, pyridoxine, folate, and vitamins A, C and E all interfere with immune function (Fabris et al., 1988; Falutz et al., 1988). Severe weight loss can also result in organ damage, which may increase the risk for fatal outcome from infections (Mahan & Escott-Stump,2000).

1.3 The role of vitamin A in HIV/AIDS

The role of nutrition in the management of HIV/AIDS infections has been researched by Semba (1994), Semba et al. (1993), Tang et al. (1993) and others (reviewed by Kennedy et al., 2000). Together with other studies their work has indicated a relationship between specific nutrient intakes and the progression of the disease. Vitamin A plays a direct role because it contributes to the maintenance of immunity. As shown in Figure 1.1 below, a vicious cycle exists between micronutrient deficiencies and HIV. The first defense mechanism against infection is an integral cell membrane (Coutsoudis et al., 1999). In cases where there are low dietary intakes of vitamin A rich foods, serum levels of the vitamin will be low and consequently the cell membranes will lose their integrity and the antigens such as the HIV can easily penetrate the membrane.

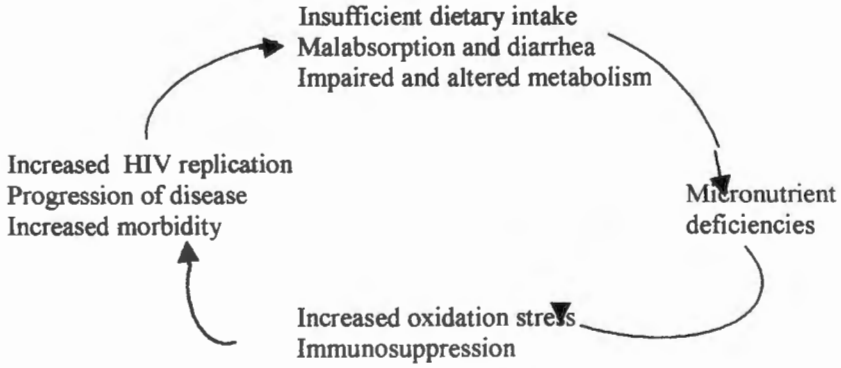


Figure1.1 Vicious cycle of micronutrient deficiencies and HIV (Adapted from Semba and Tang, 1999)

Only a few studies have been conducted in the developing countries on the effect of nutrition intervention on cases of HIV/AIDS. A cross-sectional study was carried out in the Free State province of South Africa among HIV-1 seropositive patients. Findings demonstrated less than normal values of plasma retinol (even among patients with a CD4 lymphocyte count over 500), which might be due to the low intake of vitamin A (Van Staden et al., 1998). Baum et al. (1993) indicated that the mucosal lining can be damaged as a direct consequence of HIV infection and AIDS related infections (ARI), cancerous growth and HIV enteropathy, and this can result in malabsorption and diarrhea. They further mention that this in turn can result in a vicious cycle of inadequate dietary intake by those affected leading to a more deteriorating nutritional status. Insufficient dietary intake, malabsorption, diarrhea, impaired storage, altered metabolism and increased excretion can contribute to the development of micronutrient deficiencies (Semba & Tang, 1999; Jolly et al., 1997). Work by Tang et al. (1993) indicated a U-shaped relationship between total vitamin A intake and progression to AIDS amongst HIV-1 infected men in Washington. With intakes in both the lowest and highest quartiles the progression rate was high, but with the middle two quartiles of intake the progression to AIDS remained slower. However, a lot of controversy still surrounds the issue on effectiveness of vitamin A in delaying progression of the HIV. Consequently other scientists such as Kennedy et al. (2000) suggest that "further research should be done to clarify the potential clinical application of supplementation using vitamin A" because trials that have been conducted to-date have not given consistent evidence on the benefits of this micronutrient.

1.4 Motivation for the study

Food assistance is one of the most critical needs of people living with HIV/AIDS (Hall, 1989). In a needs assessment conducted in New York City (Health Systems Agency, 1993) food and nutrition were ranked second by HIV-positive clients and third by HIV/AIDS service providers as essential service needs. In Africa, where preventive and curative interventions are difficult to implement due to limited resources, availability of a staple food providing for the needs of HIV/AIDS patients of all ages and all stages of the disease is critically important.

The staple food in the diet of South African children (Labadarios, 2001) and adults (MacIntyre, 1998) is maize meal. A potential limitation of the use of maize meal in nutrition intervention of HIV/AIDS patients is the marginal quantity and quality of maize protein, with limited amounts of lysine, threonine and tryptophan (Mahan & Escott-Stump, 2000). Soybeans, on the other hand, are a source of quality

vegetable protein, with a Protein Digestibility Corrected Amino Acid Score (PDCAAS) close to one, the same score as that of casein and egg protein (Whitney et al., 1998). The protein content of the soybean is among the highest of all vegetable protein sources. It contains 38% protein (Whitney et al., 1998). Soybean saponins have been investigated for their antiretroviral activity on HIV in vitro (Nakashima et al., 1989). Furthermore, the isoflavones in soy enhance the immune function in mice when fed in high doses (Zhang et al., 1997).

Therefore, an instant soy-maize porridge, developed and evaluated for sensory acceptability by Specialised Protein Products (SPP, Potchefstroom) was evaluated in this study for improving the general well-being and delaying progression from HIV to AIDS in a randomised controlled nutritional intervention trial. The question asked in this study was whether supplementation of the diet of HIV/AIDS patients with the instant soy/maize meal (SMM) porridge providing 3612 μgRE per 300g will improve the vitamin A status and delay progression of the disease, thereby improving their quality of life. Serum retinol and retinol binding protein (RBP) were used as markers of vitamin A status. Kennedy et al. (2000) suggest that this protein should be used as an indicator because it is a sensitive marker which is affected even during the early stages of malnutrition. HIV infection progression was assessed by the increase of CD4 count, and viral load. Anthropometric measurements used as nutritional status markers were body weight, body mass index (BMI) and lean body mass.

If an improvement would be seen then it would be an indication of reduced stress on the body due to less infections and adequate serum and liver vitamin A levels to support the immune functions of the body.

1.5 Objectives

1.5.1 Overall aim

The main purpose of this study was to assess in a randomised, placebo-controlled trial the effect of nutritional intervention over a six month period using an instant soy maize meal supplement (SMMS) containing vitamin A (300g would provide 42% of the RDA) and placebo capsules for HIV-infected/AIDS African patients in the North West and Gauteng Provinces in South Africa.

1.5.2 Specific aims

The specific aims for the study were:

1.5.2.1 To analyse the dietary vitamin A intakes of the subjects consuming the SMMS and the subjects receiving placebo capsules.

1.5.2.2 To determine the serum retinol levels of the subjects.

1.5.2.3 To compare the rates of progression of HIV as reflected by CD4 in subjects in the SMMS group with progression rates in the control (placebo) group.

1.5.2.4 To assess the effect of the supplement intake on the anthropometric measurements [body mass index (BMI) and lean body mass] of the subjects.

1.5.2.5 To test if a relationship exists between serum vitamin A, RBP, and dietary vitamin A intakes.

1.5.2.6 To identify if an association exists between dietary vitamin A intakes, the viral load and CD4 counts of the study subjects.

1.6 Structure of the mini dissertation

This mini-dissertation consists of five sections. Following the introductory chapter, a review of the literature is presented in Chapter 2. This chapter provides background information on the global and South African prevalence of vitamin A deficiency in HIV/AIDS patients and gives an overview of the causes, consequences and interventions to address these. The methodology used in this study is described in detail in Chapter 3, including the original study proposal, the study population and the process for selecting the study subjects, the methods used for data collection on dietary intakes, anthropometric measurements, quality of life assessments, and the collection, preparation, and storage of blood samples. The methods for analysis of all the variables and statistical tests used to test for significances and associations amongst variables are also included in Chapter 3, together with limitations/constraints, strengths and relative costs of using the soy maize meal supplement compared to anti-retroviral medication.

Chapter 4 reflects the results of the study relative to the study objectives. The results are presented according to the baseline and final findings after six months. These are displayed on descriptive tables, and graphs. Charts describing associations between variables are also used. The chapter further presents significance of test results performed. The baseline findings of the study are based on the viral load,

blood retinol levels and the status of the immune defense markers (CD4, CD8 as % of the lymphocytes), body mass index (BMI), lean body mass and fat mass. All these variables are compared to reference values, reported dietary vitamin A intakes (based on the food frequency method) and the quality of life. The final evaluation results are described and are compared to baseline measurements, to evaluate the effect of the intervention after the six months trial.

The results emanating from this study are discussed in Chapter 5, compared and contrasted to recent similar studies and reviews on the role of vitamin A in HIV/AIDS. Conclusions are drawn on the effect of the supplement on the vitamin A status of the subjects and the associations seen on vitamin A status, immune function markers and HIV progression. These conclusions (Chapter 6) are based on the results of the study. In this chapter recommendations are also made on relevant strategies that can be employed to control and manage the rate of HIV/AIDS progression, using nutrient supplements, and areas that require further research work in the area of vitamin A and HIV/AIDS infections.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Micronutrient deficiencies are the most commonly occurring public health concern in the developing countries (Friis et al., 2001). The most vulnerable groups for these deficiencies are children, mothers, the elderly, and persons suffering from infectious diseases. Vitamins A, B_6, B_{12}, C and E, carotenoids, magnesium, zinc and selenium play an important role during infectious diseases, whereby similarly with energy providing macronutrients, the body utilises more of the nutrients as part of the defense mechanism to strengthen the immune system (Friis & Michaelsen, 1998; Semba & Tang, 1999; Tang et al., 1993). Studies conducted among HIV infected people found an association between prevalence of vitamin A deficiency and lower CD4 cell count (Lacey et al., 1996; Semba et al., 1996). Furthermore vitamin A deficiency has been associated with an increased susceptibility to infectious diseases and mortality (Jolly et al., 1997; Kennedy et al., 2000; Semba et al., 1993). The international nutrition programmes have therefore identified vitamin A as one of the nutrients to be extensively researched on its effectiveness in improving the nutrition profiles of people infected with HIV (Cervinskas & Lotfi, 1996). The following sections will review studies that have found vitamin A deficiency to contribute to disease progression and the health consequences suffered by those with HIV infection. Because this nutritional intervention study included only non-pregnant adult HIV/AIDS patients the focus of the literature reviewed will be on male and non-pregnant female HIV-infected persons.

2.2 Global prevalence of vitamin A deficiency

The report from the Micronutrient Initiative (MI) information paper (Cervinskas & Lotfi, 1996) indicates that vitamin A deficiency (VAD) is widely prevalent, especially in the developing countries, where the World Health Organisation (WHO) estimates that 228 million children are sub-clinically affected, either severely or moderately, while more than three million have clinical VAD. Sub-clinical VAD prevalence assessed by serum retinol levels show that 75 to 140 million children are affected in the developing countries. This deficiency is a problem in 75 countries. The MI therefore notes that adequate vitamin A status is important for maintenance of good health and disease prevention, where the benefits of supplementation would play an important role as an attempt to prevent consequences of VAD.

2.2.1 Vitamin A deficiency in the region.

Between a third and a half of the children in Southern Africa are vitamin A deficient (De Wagt, 2000). The 1994 South African Vitamin A Consultative Group (SAVACG) study among children 6 to 71 months found a 33% national prevalence of marginal vitamin A deficiency. In 1999 the South African National Food Consumption Survey (NFCS) conducted among children aged 1 –9 years found that the intake of 69% of children did not meet two thirds of the RDA, where the median intake for the group ranged from 7 to 24% of the RDA (Labadarios, 2001). To address the prevailing problem of vitamin A deficiency South Africa decided on a combination of multi strategies which will also focus on food fortification for vulnerable groups, in which academic and research institutions are expected to provide technical support (De Hoop & Matji, 2000).

2.2.2 HIV/AIDS prevalence

The Transition, Health and Urbanization in South Africans (THUSA) study (Vorster et al. 2000) conducted from 1996 to 1998 among "apparently healthy" subjects in the North West province, 13% men and 11.6% women were tested HIV positive. Both these groups had adequate serum retinol at a mean of 46 μg/dL for men and 42μg/dL for women.

2.3 Causes of vitamin A deficiency

In an ideal situation vitamin A would be provided by the diet in small regular amounts that are in balance with other essential nutrients (Mahan & Escott-Stump, 2000). The body treats large doses of vitamin A in a special manner. It would use the vitamin to perform physiological roles and excess would be stored in the liver to be used in times of need, when dietary intakes are not adequate to meet these requirements. However, the rate at which the stores will disappear will be directly proportional to the liver stores. The stored vitamin is rapidly used until a critical level of the stored vitamin A is reached, then the rate of depletion slows down (Augustus, 1997). Semba et al. (1996) mention that vitamin A deficiency during infections may be due to a number of factors including reduced intakes and malabsorption of dietary sources, loss of appetite, dysphagia, medications, depression, socio-economic factors, food aversion, oral and oesophageal candidiasis, nausea, vomiting and chronic fatigue, causing less time spend on efficient procurement, preparation and consumption of nutritious food (Semba & Tang, 1999). Augustus (1997). According to Stephensen et al. (1994) and Friis et al. (1996) the utilisation of vitamin A and urinary losses are increased during infections.

2.3.1 Reduced food intakes

Fever, opportunistic infections and painful mucosal lesions in the mouth can affect the taste buds, and together with cancers of the pharynx and/or oesophagus, could contribute to the poor appetite (Smith et al., 1992; Augustus, 1997). The low dietary intake can also occur in asymptomatic HIV patients consequently leading to loss of weight (Semba & Tang, 1999).

2.3.2 Malabsorption

Malabsorption of vitamin A can be caused by bacterial overgrowth in the digestive system due to poor functioning of the mucosal lining and failure to secrete acid (Smith et al., 1992). Semba and Tang (1999) also attribute malabsorption of vitamin A to poor absorption of fats, because the chemical structure of the vitamin is lipophilic, hence it is affected in times of fat malabsorption.

Goblet cells, immune function cells (lymphocytes and phagocytes), and harmless bacteria have a role in maintaining and promoting immunity, and all these are either found on the surface of the microvilli or embedded within the lymph duct in the gastrointestinal membrane lining (Whitney et al., 1998). These authors mention that goblet cells secrete mucous which forms a slippery surface on the microvilli, making it difficult for harmful microbes to attach to the surface of the intestines. Similarly, the protective microbes that exist on the surface of the intestinal lining compete with harmful organisms and produce short chain fatty acids that will prevent the latter from attaching to the surface of the intestines. They also mention that immunoglobulin A, produced by some of the lymphocytes (B-cells), affects most of the immune functions in the intestines. In malnourished persons and those experiencing stress, such as infections, damage can occur to the intestinal lining where the microvilli is located. Consequently the structure of the villi is affected, as well as the function of the lymph tissue, as the intestinal villi shrinks and its capacity to digest and absorb nutrients is impaired, the harmful organisms are able to penetrate into the intestinal surface. The reduced blood flow to the intestines and the inadequate nutrient supply can cause the deterioration of the intestinal cells (Whitney et al., 1998).

The intestinal wall is therefore altered in HIV/AIDS subjects, resulting in malabsorption. The condition in turn causes diarrhea, consequently leading to low nutrient absorption, and people affected reduce their dietary intake to avoid further occurrence of diarrhea (Grunfeld & Feingold, 1992).

2.3.3 Impaired storage

The liver is a site for storage for many micronutrients, including vitamins A and E and iron. Hepatitis B and C are extremely common in HIV-infected adults (Semba & Tang, 1999). Thurnham (1989) suggests that the reduced levels of serum retinol in HIV/AIDS patients could be a result of the reaction of acute phase proteins, where retinol bound to RBP flows into the extravascular space during infections. Literature on studies done on children indicates that high level vitamin A dosing does not guarantee that absorption rates will be high. Contrary to this, the efficacy of absorption is hampered and children with infection will absorb less vitamin A, relative to higher absorption rates in normal healthy children (Stoltzfus & Klemm, 1997).

2.3.4 Altered metabolism

In HIV infected patients the risk of developing renal diseases is increased (Semba & Tang, 1999). These include acute renal failure, fluid-electrolyte and acid-base disturbances, HIV-associated nephropathy and other glomerulopathies. During all these conditions losses of RBP and albumin may occur, leading to loss of vitamin A. In response to the acute phase of infection, RBP dissociates from the transthyretin-retinol binding protein-retinol complex, resulting in loss of this acute phase response protein in the urine (Stephensen et al., 1994; Jolly et al., 1997). In their study Jolly et al. (1997) also found an association between greater urinary retinol loss with body weight loss and death.

2.4 Dietary intakes by different socio-economic groups in South Africa

A study conducted by MacIntyre et al. (2001) amongst the African population in the North West Province of South Africa, revealed that for some groups the reported vitamin A intakes were close to or above the Recommended Dietary Allowance (RDA) by FAO of 600 and 500 μgRE for men and women respectively (see Table 2.1). The above situation is a probable indication that all of the men and women in all stratum groups (the farm, rural, informal settlements, and the middle and upper class urban) consumed adequate amounts of vitamin A when compared to the FAO (Food and Agriculture Organisation) recommendations. However, in comparison to the United States recommended dietary allowances (USRDA), the intake of the farm workers was inadequate.

Table 2.1 Vitamin A intakes among stratified groups in the THUSA study

Study subjects	MEAN DAILY DIETARY VITAMIN A INTAKES IN RETINOL EQUIVALENT (RE)						
	USRDA	FAO	Rural	Farm	Informal settlement	Middle class urban	Upper class urban
Females							
Mean	800	500	573	533	773	829	1246
Males							
Mean	1000	600	609	588	729	762	900

Adapted from MacIntyre et al., 2001

USRDA= United States recommended dietary allowances for populations aged 25 to 45 years.
FAO = Food and Agriculture Organisation safe limits.

A meta-analysis of literature by Vorster et al. (1997) showed that the mean daily vitamin A intakes of Africans in urban areas in South Africa aged 15 to 24.9 years were 373 and 452 RE in males and females respectively, while for the older age group (25 to 64.9 yrs) the mean intakes were 640 and 563 RE respectively. Lubbe & Maree (1973) also indicated that the mean intakes for the rural populations aged 20 to 50 years and above were 662 RE, which was 82% of the RDA. Hence it is assumed that using SMMS in this study will top-up the RDA as indicated earlier in this chapter, and help boost the immune system, by providing the vitamin A which contributes to cell differentiation, and thus assist in boosting the CD4 count and controlling the rate of progression of HIV.

2.4.1 Cultural practices in cooking and preparation of vitamin A rich food

The richest sources of preformed vitamin A are foods of animal origin namely liver, milk and milk products, butter, and eggs (Whitney et al., 1998), which may be too expensive for some Africans. Cultural taboos often exclude micronutrient-rich foods and may also contribute to deficiencies (Hanson, 1996). Many vegetables and some fruits contain provitamin-A carotenoids. However, the most commonly used methods for processing and cooking of vegetables which are sources of carotenoids amongst African populations also allow great losses of the nutrient which is highly oxidised and is unstable if exposed to ultraviolet light. Boiling vegetables for a prolonged period, frying and exposing the vegetables to light and air after they have been peeled or cut can deteriorate the levels of carotenoids (Hanson, 1996).

2.4.2 Dietary patterns including fat and protein intakes

The rate of absorption of dietary vitamin A is influenced by both the quality and quantity of dietary fat because it is fat soluble. The vitamin is also transported from the intestines to the liver as retinyl palmitin attached to chylomicrons. From the liver it circulates in the blood as retinol, attached to RBP, a specific transport protein which forms a complex with plasma prealbumin (Whitney et al., 1998). It is therefore essential that the diet should have adequate amounts of fats and proteins to facilitate adequate absorption and transportation of vitamin A to the relevant body cells. The THUSA study report on the metabolic profiles of asymptomatic HIV positive Africans in the North West region (Vorster et al., unpublished). The study subjects were following a prudent, low-fat, high-carbohydrate diet. However, it was deficient in micronutrients, and vitamin A was one of the nutrients notably taken in insufficient amounts (see Table 2.1). Dietary fat provided <30% of the total energy in the diets of Africans in the urban area, and for those in the rural areas fat provided 20% of the total energy (MacIntyre et al., 2001). In the meta analysis of the nutritional status of South Africans, Vorster et al. (1997) report protein intakes between 15% and 20% of the total energy, with plant protein contributing more than animal protein in diets of the African study population. MacIntyre et al. (2001) noted mean protein intakes of 65g in the rural areas and 57g in the farm setting. Intakes in the urbanised groups were also above the RDA. It therefore seems as if the intake of nutrients required for the transportation of vitamin A were adequate even though intakes of the vitamin was limited among the population that the present study sample was drawn from. As indicated above even when dietary intake of vitamin A is within the RDA, serum levels of the vitamin in the asymptomatic HIV-infected individuals was found to be lower than their control counterparts (Vorster et al., unpublished). Factors that could contribute to this may be that the utilisation and excretion of vitamin A are increased during HIV- infection (Kennedy et al., 2000; Semba & Tang, 1999), hence the need to increase the intakes for those affected.

2.5 Consequences of vitamin A deficiency in HIV/AIDS infection

Kennedy et al. (2000) in their review mention that in vitro and clinical studies suggest that an interrelationship exists between vitamin A and HIV disease progression, mortality and viral replication of HIV-1, following quite complex mechanisms. These authors cite evidence that malabsorption and diarrhea are common during HIV-1. Table 2.2 below reflects the results of the intervention studies where the effect of vitamin A supplementation was assessed on different clinical outcomes.

In persons with infectious disease, vitamin A deficiency predisposes the subject to be more susceptible to attack by other diseases, and this results in a vicious cycle, where the subject is not able to metabolise and utilise nutrients effectively including dietary pro-vitamin and vitamin A (Kennedy et al., 2000). People suffering from chronic infections such as HIV/AIDS will have significantly lower liver vitamin A levels, relative to subjects with non-infectious chronic disorders, due to several mechanisms (Kennedy et al., 2000). In response to the infection vitamin A is used for activation of acute phase response, preparing the host to repair the damaged tissue in reaction to the injurious stimuli. This response can be prolonged causing a deterioration in the nutritional status of the affected individual (Vitamin Information Centre, 1998). Stores of the vitamin are then gradually depleted during shortages resulting in reduction of circulating vitamin A with consequence of malabsorption due to reduced integrity of the epithelial lining. The requirement of vitamin A is then increased in HIV/AIDS. Kennedy et al., (2000) also indicate that the depletion in serum retinol levels is more marked in symptomatic HIV/AIDS patients even when their intake of this vitamin is well above the RDA. Tang et al. (1993) note that animal and human studies have confirmed that a deficiency in vitamin A can also inhibit humoral antibody formation and in their study they found an association between this vitamin and progression to HIV.

With HIV/AIDS patients the virus replicates within the host cells, and the body's immune system has to destroy these infected cells (Peakman & Vergani, 1997). The CD4 cells play an important role in this process, their surface have a receptor for the HIV, hence the virus manages to penetrate and destroy them, then a significant decline in the CD4 count occurs with progression of the disease (Peakman & Vergani, 1997). These authors cite a study conducted in San Francisco among a cohort of asymptomatic patients with a CD4 count of <400 cells/mm^3 and a 50% progression rate to AIDS in three years. They further observe that recent studies reveal a 40% progression rate among patients with CD4 counts of <200 cells/ mm^3 within a year. Nutritional status may have a direct effect on the progression of HIV. Semba et al. (1996) identified an association between vitamin A deficiency and HIV-type 1 infection, where the mean CD4 cell counts of vitamin A deficient (<1.05 μmol/L) adults was <200 cells/mm^3 compared to adults with normal vitamin A levels who had a mean CD4 cell count of >200 cells/ mm^3.

Table 2.2 Prospective studies examining the effect of vitamin A deficiency on HIV-1 disease progression

Study	Country	Sample size	Serum retinol exposure	Outcome	Measure of association	95% CI/*p*-value	Comment
Abrams et al., 1993	United States	296	>28k IU/day	CD4<500 cells/dL, progression to AIDS (6 years)	RR = 0.63	(0.46-0.87)	Adjusted for age, smoking, symptoms, and total energy intake
Baum et al., 1995	United States	108	<1.05μmol/L	Change in CD4 cell count	$\beta = 3.59$	$P=0.02$	Adjusted for ART use
Burns et al., 1999	United States	449	<1.05μmol/L <0.07μmol/L	Low birth weight	RR = 4.58 RR = 6.99	(1.57 – 13.4) (1.09 – 45.0)	Adjusted for BMI and CD4 cell count
Camp et al.,1998	Rwanda	30	Mean retinol levels	HIV-1 RNA	$r = -0.48$	$P=0.04$	
Rich et al.,2000	United States	122	<1.05μmol/L	Infant disease progression by 8 months		$P=0.05$	Univariate analysis
Semba et al., 1993	United States	179	<1.05μmol/L	Time to death within 22 months	ROR = 4.3	(1.1 – 17.8)	Adjusted for age, CD4 cell count, haemoglobin, haematocrit, platelet, and hepatitis B
Semba et al.,1995[a]	Malawi	474	<1.05μmol/L	Infant mortality	RR = 1.93	$p = 0.0001$	Adjusted for CD4 cell count, CD8 cells, and CD4/CD8 ratio
Semba et al., 1995[b]	United States	290	<1.05μmol/L	Time to death within 28 months	OR = 4.55	(1.83 – 11.31)	Adjusted for CD4 cell count and BMI
Semba et al., 1997	Malawi	467	<1.05μmol/L	Child's growth	RR = 2.30	$p = 0.004$	Adjusted for child's age, sex, HIV-1 status, and maternal BMI
Tang et al., 1993	United States	281	>20 268 IU/day 9062 –20 268 IU/day <9062 IU/day	AIDS	HR = 0.95 HR = 0.57 HR = 1.00	(0.54-1.69) (0.35-0.91)	Adjusted for age, symptoms, CD4 cell count, energy intake, ART use, and PCP prophylaxis
Tang et al., 1996	United States	281	>11 179 IU/day 7621-11 179 IU/day <7621 IU/day	Time to death within 8 years	HR = 0.82 HR = 0.60 HR = 1.00	(0.52-1.28) (0.37-0.98)	
Tang et al., 1997	United States	311	≥3.15μmol/L 2.45≥μmol/L<3.15 1.82μmol/L<2.45 <1.82μmol/L	First AIDS Dx	HR = 0.73 HR = 0.94 HR = 1.13 HR = 1.00	(0.46-1.16) (0.60-1.47) (0.73-1.75)	Adjusted for HIV symptoms, CD4 cell count, age, ART use, alcohol consumption
Ullrich et al., 1994	Germany	149	<0.88 μmol/L	Diarrhoea	ROR = 1.6	$P < 0.02$	Adjusted for liver dysfunction and fever

Adapted from Kennedy et al., 2000

CI = confidence interval; r = correlation coefficient; ROR = risk odds ratio; HR = hazard rate; ART = antiretroviral therapy; PCP = pneumocystis carinii pneumonia; OR = odds ratio; BMI = body mass index (kg/m^2); β = beta coefficient obtained from linear regression; and RR = relative risk; $p < 0.05$ statistically significant; *p*-value obtained from the studies.

2.5.1 Vitamin A and Karposi's sarcoma

Vitamin A can mediate prevention of cancer in HIV through two mechanisms. The first is directly involved with the T-lymphocytes and the second is mediated through cell differentiation (Semba, 1998).

The Centers for Disease Control (CDC) has classified HIV-infection into four groups according to the combination of manifestations and complications of the immune deficiency system. The fourth group of the condition is AIDS defining, which is further catagorised into five stages. Group IVB is characterised

by neurological disease, IVC by opportunistic infections, IVD by secondary cancer and IVE by other complications (Peakman & Vergani, 1997). The normal levels of lymphocytes are reduced in HIV/AIDS, hence the absolute numbers of CD4 T lymphocytes are used as the index for the severity of the disease, where the levels for the asymptomatic patients are lower than those presenting symptoms. CD8 lymphocytes are affected in the latter stages of the infection. The absolute count increases two to three fold during the initial course of the disease, but decline in the latter stages (Peakman & Vergani, 1997). These authors explain that some of the specific markers that are involved with cancer and vitamin A are CD8 lymphocytes, and natural killer cells (NKC). The CD8 lymphocytes are cytotoxic and require vitamin A for their activation, they are also involved in lysing virus infected cells, while the NKC are involved with antitumour and antiviral immunity, and vitamin A deficiency has been found to reduce their effect (Semba, 1998). Supplementation with high doses of the vitamin increases the number of circulating NKC in children with AIDS (Hussey et al., 1996).

Vitamin A is found in somatic cells in the form of retinoic acid. It is attached to deoxyribonucleic acid (DNA) by receptors and regulates the expression of genes for maintenance of epithelial tissue and guides differentiation of other tissues (Semba, 1998). Simple columnar epithelium is found in the intestinal mucosa, while the squamous stratified keratinising epithelium exists on the epidermis, which serves specific purposes in the body (Semba, 1998; De Luca et al., 1994). The presence of epithelial tissue in the body is regulated by hormones and dietary vitamin A. Severe deficiency of vitamin A can result in formation of stratified squamous epithelium, which can eventually keratinise and form cancerous cells either in the lungs, skin, colon, breast, prostate, cervix, bladder or oesophagus. Vitamin A can also be used as a chemopreventive agent in formation of skin cancer, where 30μg of retinoic acid or 600μg of β-carotene per gram of diet would inhibit the formation of carcinoma (De Luca et al., 1994).

2.6 Randomised controlled trials of vitamin A supplementation among HIV-infected individuals

Kennedy et al. (2000) reviewed studies in the MEDLINE that were conducted from 1980 to 2000 to assess the effect of vitamin A on disease progression and immune status. Their review on the effect of vitamin A on diarrheal-related morbidity, CD4 count and HIV-1 viral load will be discussed below.

2.6.1 Effect of vitamin A supplementation on diarrheal-related morbidity and mortality

Studies have been undertaken to assess the effect of vitamin A on diarrhea related morbidity and mortality among children and adults infected with the HIV – 1 (Kennedy et al., 2000). Some of these studies

showed a positive effect while others did not have enough statistical power to detect any significant differences. In an intervention study conducted among children with HIV-1 infection in South Africa large doses of vitamin A reduced the frequency of diarrhea significantly compared with children without the infection (OR = 0.51; 95% CI = 0.27- 0.99). In Tanzania supplementation trials among children also found that the supplement significantly reduced the risk of mortality due to diarrhea (RR = 0.07; 95% CI = 0.00 – 0.49). Kennedy et al. (2000) also cite two studies that were conducted among women, on the effect of vitamin A on diarrheal morbidity. No significant change occurred in a study with 106 HIV-positive Zambian adults with diarrhea-wasting syndrome who were randomly given a vitamin A supplement together with other micronutrients or placebo (RR = 0.99; p = 0.98). Nor did the study conducted among 312 pregnant women in South Africa show any effect of β-carotene on diarrhoea (RR = 0.34; 95% CI = 0.08-1.32).

2.6.2 CD4 cell count

Both *β*–carotene and vitamin A have been used in intervention studies among HIV infected adults to assess its effect on the CD4 cell count (Kennedy et al., 2000). One study conducted in the United States with 21 HIV-1 seropositive adults found significant results in total white cell blood count, CD4 cell count and CD4/CD8 ratio. A larger sample of the same study did not find any significant association between the supplement and improvement in the above mentioned cell counts. In this latter study the subjects were randomly assigned to the *β*–carotene or placebo group. All subjects were given multivitamins, which are assumed to have masked the effect of *β*–carotene. A double-blind, placebo-controlled study conducted in Tanzania with 1057 HIV infected women, who were given either vitamin A, placebo and multivitamins without vitamin A or with vitamin A, and multivitamins with vitamin A also failed to give any significant results on improvement of CD4, CD8 and CD3 cell counts and vitamin A supplementation (Fawzi et al., 1998).

2.6.3 Viral load

In their review Kennedy et al. (2000) mention that studies failed to find a significant association between HIV-1 viral load and vitamin A supplementation. These authors assume that results in the two studies reviewed may have been affected by the bias that most subjects were not clinically vitamin A deficient (serum retinol levels <1.05 μmol/L) at baseline. Coutsoudis et al. (1997) did not find any association

between vitamin A supplementation and viral load in 24 HIV-1 positive pregnant women, even though subjects in their study were vitamin A deficient.

2.7 Cost of vitamin A dietary sources

The micronutrient RDA's for the general public are not adequate to meet the requirements of HIV/AIDS patients. Semba and Tang (1999) state that the RDA is the amounts of dietary intakes for an essential nutrient and these levels are considered to be adequate to meet the known nutrient needs for almost all healthy persons, based on scientific knowledge. They further mention that the RDA is two levels above the standard deviation, consequently even if a healthy person consume less than the RDA it is possible that the intake would still be adequate. However, for HIV/AIDS patients it is possible that their needs are elevated due to the factors mentioned in section 2.2.1-5 above. It is therefore necessary to ensure their intakes are adequate by providing supplements as a short-term measure. In the meantime the policy makers and relevant programmes should promote and support further research to establish the RDA's for those presenting the condition and food based interventions, because they are cost effective, culturally acceptable and ensure sustainability.

Food fortification is another effective, economical, flexible, feasible and socially accepted strategy to ensure increased nutrient intake. South Africa is already working with several international agencies on fortification of commonly consumed foods (De Hoop and Matji, 2000). Recommendations made at the Commonwealth Regional HIV/AIDS and Nutrition Workshop in Maputo in February 1999, emphasized that the Commonwealth Secretariat for Health should facilitate dissemination of models of good practice in HIV/AIDS and nutrition. Hopefully these will include recommendations to address micronutrient deficiencies (Cervinskas & Lotfi, 1996). The Department of Health in South Africa is already in the process of developing information, education and communication (IEC) materials. These will be based on the country's nutrition guidelines for people living with TB, HIV and other illnesses such as diarrhoea, constipation, heartburn, and will address food safety and food selection.

The cost of daily consumption of 300g of SMMS (SD 605) is compared to antiretroviral medication (Vorster, H. 2001) in Table 2. 3. At R75. 60 per month it is therefore assumed that this food product could be viable for economically disadvantaged HIV persons.

Table 2.3 Comparison of monthly cost for SMMS and anti-retroviral agents

SMMS for one month	**Antiretroviral agents for one month**
*SD 605 300g/day @ R2.52 R75. 60	AZT 200 mg t.i.d. R39 595. 50 Saquinavir 800 mg. R111 500. 00 Lamivudine 150 mg t.i.d. R76 530. 00 Hivid 150 mg t.i.d. R34 790. 00 Didanosine t.i.d. R29 966. 40 Hydroxyurea mg bd. R10 781. 58
	Standard "Optimal treatment" AZT + lamivudien + saquinavir = R227 629. 50
	The "poor man's option" Didansine + hydroxyurea = R40 747. 98

Adapted from Vorster, H., (2001)
*SD 605 = Brand name for the SMMS

2.8 Summary

From this literature review, it is obvious there is a need for researchers in the area of nutrition in the Southern Africa region, to extensively investigate and identify cost effective, easily accessible functional food commodities, to be used effectively in the management of HIV infections and other related health problems. To date the soy bean and its products are already being extensively promoted in the region, for their benefit on soil fertility and other health benefits that will not be discussed in this paper. It therefore lies with the experts in the area of nutrition to justify its merits.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Introduction

This multi-disciplinary placebo controlled intervention trial was undertaken by researchers from Potchefstroom University for Christian Higher Education (PU for CHE) from April to October 2000. Participants were African HIV/AIDS volunteers at all stages of HIV infection in the health catchment area of Ventersdorp, Makwassie, Fochville, and Potchefstroom within the North West province of South Africa.

The research proposal was approved by the Ethical Committee of PU for CHE (approval number 99M04), and the North West Department of Health and Developmental Social Welfare granted the permission to proceed with the study (Appendix A).

3.2 Study design

3.2.1 Organisation of the study

The study consisted of baseline, middle (three months later) and end (six months after baseline) evaluations as shown on the organisation chart on Figure 3.1 below. During these three stages six stations were set up, each study subject passed through all the stations using the station card attached as Appendix B, which was signed by the researchers on completion of each assessment session.

During the baseline, at midterm review and final evaluation, assessments were done on the dietary intake, anthropometry, biochemical, clinical symptoms and signs, sensory and quality of life variables as shown in Figure 3.1.

3.2.2 Subjects

The research was initially designed to be a parallel double-blind study with a total sample size of 100 subjects. The total number of subjects who volunteered came up to 104. However, because of the risk of exposing their HIV status and other unexplained reasons some of the clientele that had signed consent forms to participate in the study did not turn up for the baseline measurements. At baseline 78 volunteers attended. In July when the midline measurements were taken the numbers had further declined, some of

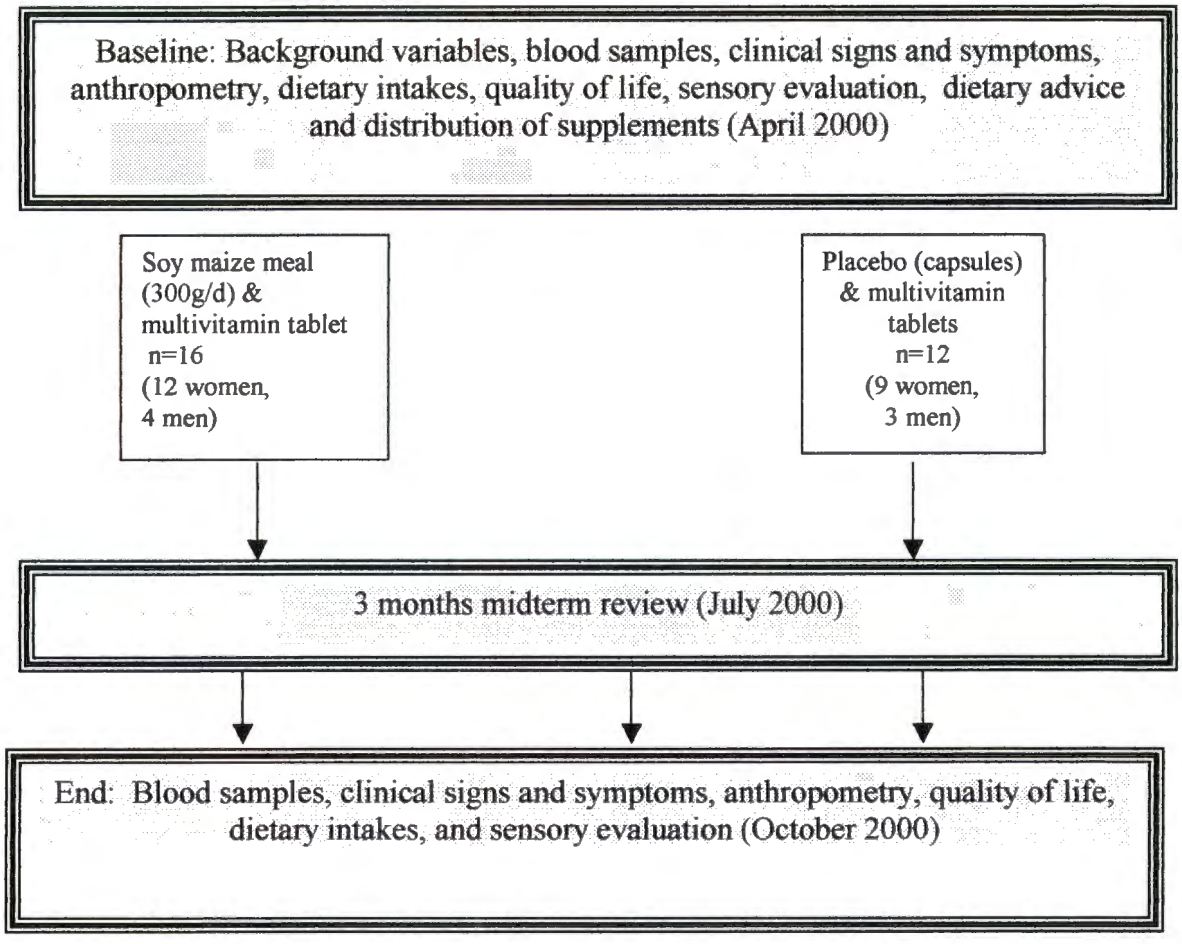


Figure 3.1 Study design

the subjects on the capsules had failed to turn up for set appointments for collecting their capsules, one subject on the soy maize meal supplement (SMMS) withdrew because of job commitments, another one was not able to take this particular type of supplement into her home incase her family would question her about it (she wanted to maintain confidentiality) and three dropped out for unknown reasons. One subject that experienced nausea from eating SMMS was moved to the phytochemicals group (not reported in this dissertation). Two other subjects stopped participating due to severe illness (one of them died before completion of the study). At the final assessment a total of 52 subjects remained in the study.

The original protocol included a control group for the SMMS treatment consuming pure instant maize meal. However, the design had to be modified because of the reasons mentioned above, hence it was decided that only three groups would be included [one group was allocated to the SMMS supplement, the second group was to take the phytochemical (antioxidant) capsules and the third group would be the control taking placebo capsules]. The double blind component for the soy group was thus eliminated.

Fourty nine (11 men and 38 women) subjects completed the study, including 16 in the SMMS group, 13 in the control group and 21 in the antioxidant group.

3.3 Study population

The age group of the study subjects ranged from 21 to 45 years, mainly because they were able to give their own consent to participate in the study. Current statistics show that this age range is the one that is affected most by the HIV/ AIDS pandemic as reflected by the results from a survey that was done amongst women attending antenatal clinics in the North West province of South Africa in 1998 (Department of Health, North West Province, 1998). The exclusion/inclusion criteria for the study are presented in 3.4.2. To participate in the study subjects had to fall within the age group mentioned above, and had to be HIV positive. An HIV test was performed on the subjects to confirm the status and counselling was provided pre- and post-testing. Subjects were asked whether they preferred to maintain confidendiality or would they be willing to participate in group sessions. The subjects were allocated to the different study groups, based on their proximity to the University. Those living in the Potchefstroom, Ventersdorp and Rysmierbult farm area were put in the SMMS group. It would be more convenient to distribute the product to them every two weeks and monitor compliance. The subjects from other areas were randomly allocated to either the placebo (control) or the antioxidant group.

3.4 Sampling procedure

3.4.1 Sample selection

All the participants in this study were conveniently recruited from the outpatient clinics of the respective health facilities, namely Gateway, Boiki Klapi, Mohadine, Pothefstroom, Makwassie, Wedela, Fochville and Ventersdorp clinics and farm workers from Castello and Rysmierbult farms in the Potchefstroom district. The recruitment was done from January to March 2000. On recruitment the purpose of the study was explained and the volunteers gave written consent to participate. Pre-counselling (before the HIV test was done) and post-counselling were provided by members of the research team who were trained counsellors (MM, CW and AR). Venous blood samples were drawn by a registered nursing sister (CL) to confirm their HIV status using the enzyme-linked immunosorbent assay method (ELISA), confirmed with the Western Blot assay (Bio-rad, Germany). These tests were carried out by Pathcare Laboratory (Drs. De Villiers, De Beer, Strydom and Van Zyl) in Klerksdorp, South Africa.

The SMMS intervention group was recruited from clinics within the vicinity of Potchefstroom (Potchefstrooom and Ventersdorp clinics and the farm workers from Rysmierbult), because it would be more convenient considering the cost and distance to distribute the supplement on a bi-weekly bases. Both the phytochemical and control groups were recruited from clinics in Makwassie, Wedela and Fochville. When the intervention was started in April, 25 subjects were in the SMMS group, and the remaining persons were randomly allocated to either the control or the Phytogard® and Ultragard Forte® capsule group. The researchers as well as the subjects were blind to which capsules were the placebo or the phytochemicals, as their appearance was identical.

3.4.2 Exclusion/inclusion criteria

The study excluded pregnant and lactating mothers, patients that have been on antiviral medication (though at this stage none of the HIV/AIDS patients seen in the clinic visited had access to antiviral medication), and those with diseases other than HIV/AIDS such as hypertension and diabetes or on medication for tuberculosis.

3.4.3 Monitoring compliance

For control and monitoring purposes the SMMS had to be distributed bi-weekly for better compliance, to limit the chance of other members of the subjects' families taking the product, and to control for shelve life of the product. The SMMS subjects were each given food diaries (Appendix C) to record all the foods they ate for the first three months of the study, any ailments that they might suffer during that period and to monitor compliance. These were collected and checked at the end of each bi-weekly period by two of the researchers (PH, MM), when the next supply of the supplement was distributed. Where necessary these researchers gave individual or group dietary counseling to the patients who required it. The subjects taking the antioxidants or the placebo were each given a calendar (Appendix D) to mark against each capsule and tablet that they took, and illnesses that they might have experienced over that month. These diaries were collected by three of the post-graduate students (KS, WD, FL) when the subjects received their next supplies of the capsules/tablets.

3.5 Variables

3.5.1 Dietary intakes

A validated quantitative food frequency questionnaire [(QFFQ, Appendix E) (MacIntyre, 1998)] and a twenty four hour recall questionnaire (Appendix F) were filled in using a validated food portion

photograph book (Venter et al., 2000) by two Tswana speaking trained research assistants with the help of one lecturer (HV) and three post graduate students (PH, KS, and FL). The research assistants have been working in similar studies undertaken by the Department of Nutrition, PU for CHE, such as the THUSA study. They were able to administer the questions in the home language that the subjects were comfortable with, and were given a refresher training course just before the baseline measurements were taken.

3.5.2 Anthropometry

Anthropometric measurements of the subjects were taken by a post-graduate student from the School of Biokinetics, PU for CHE (RvdM), as part of her Masters studies. Body weight in kilograms (kg) was measured to the nearest 0.5 kg on a Seca® beam balance, with subjects wearing underclothes and bare footed. Height in centimetres (cm) was assessed to the nearest 0.5 cm using a Seca® stadiometer. Triceps, mid-upper arm and abdominal skin folds, and subscapular thickness measurements were taken in triplicate with a Slimguide® skinfold caliper. These measurements were used to determine the percentage body fat, lean body mass, fat mass, and the BMI was calculated using the weight in kilograms divided by height in meters squared (W/H^2).

3.5.3 Biochemical samples

Blood samples were drawn from fasting subjects from the *vena cephalica* by a registered nursing sister (CL), using a sterile butterfly infusion set (Johnson & Johnson, 21G, 19mm) and syringes. These samples were to be analysed for the following serum micronutrients: vitamins A, B_{12}, C and E, iron status (ferritin, transferrin, haemoglobin, haematocrit, iron and total iron binding capacity), serum lipid profile (total serum cholesterol, high-density lipoprotein cholesterol, low- density lipoprotein cholesterol, triglycerides), the serum proteins including acute phase proteins [fibrinogen, C-reactive proteins, and serum retinol binding protein (RBP)] which would give an indication of the infections at the time that the measurements were taken, the viral load, differential white blood cell counts (lymphocytes, neutrophils, monocytes, eosinophils, and basophils), T lymphocyte counts including CD4 and CD8 cells and $CD4:CD_8$ ratio, and platelet counts.

3.5.3.1 Sample preparation of the blood serum and plasma

Serum sample preparation

The 10 ml aliquots of the blood samples for analyses of serum variables (C-reactive protein, albumin, lipids, RBP, ferritin, transferrin, iron, total iron binding capacity and liver functions) were left to clot in sterile Vacutainer® STT® glass tubes containing a gel and clot activator, then centrifuged (Universal 16R™, HETTICH) at 3000 rpm for 15 minutes at 4°C within 2 hours. The serum was then divided into plastic micro-tubes on dry ice and then stored at –82°C (WO, DL) in a Nuaire™ biofreezer for six months, until the final samples were collected and analysed all at once.

The 5 ml blood samples that were to be analysed for micronutrients such as vitamin A, C, B_{12} and E were collected in sterile vacutainer tubes containing a gel and clot activator. They were protected from ultra violet light by immediately covering them with foil after they were drawn, as this could have destabilised the vitamins and alter the final micronutrient content values/levels. These samples were left to clot and were also centrifuged (within 2 hours) to yield serum, divided into aliquots in plastic micro-tubes on dry-ice and then stored at –82°C, until the final samples were collected.

Plasma sample preparation

Citrated blood samples were immediately centrifuged for 10 minutes in plastic tubes at 2000 rpm at 4^0C, kept on dry ice and then stored at -82^0C until the final samples were collected, when they were transported to Pretoria on dry ice.

For the determination of the viral load, a 5 ml sample of blood from each patient was transferred to a sterile K_3 EDTA glass tube. They were also centrifuged similar to the plasma citrates, and 2ml of the plasma was pipetted into sterile cyrovials using sterile RNase-free filter tips. These were then kept on dry ice and stored at -82^0C until the final samples were collected, when they were transported under the same storage conditions.

For analysis of vitamin C, 0.5 ml of the plasma was pipetted into 0.5 ml reagent, then vortex mixed for 2 minutes and centrifuged for 7 minutes. The supernant produced was then pipetted into plastic micro-tubes on dry ice and stored at -82^0C until they were ready for analysis in Pretoria with the rest of the other samples.

3.5.4 Clinical symptoms and signs

The study subjects were all assessed and asked by the nursing sister (CL) whether they had recently or presently experienced any skin rashes, malabsorption (diarrhea), mouth sores, ear infection, and cold symptoms, as listed on the demographic card (Appendix B). The blood pressure was assessed using a Tycos® sphygmomanometer and a stethoscope. Body temperature was measured from the ear using the Braun® thermoscan (Medsurg, Pretoria) with a calibrated normal range of 35.8 to 38.0 ^{0}C.

3.5.5 Sensory acceptability of the soy maize meal supplement

The SMMS was evaluated for its sensory properties, amongst all the study subjects during recruitment. It was also tested in the soy maize supplement group during the midterm review and final evaluation by one of the post-graduate students (MM). The acceptability level was high ensuring compliance. The results are reported elsewhere.

3.5.6 Quality of life

The quality of life of all study subjects was assessed by administering a validated 20 item quality of life instrument (Wissing et al., 1999, Appendix G) during the three stages of the measurements. This was administered by the trained counsellors (MM, CW and AR).

3.6 The intervention

All study subjects were given relevant culture sensitive nutrition education for HIV/AIDS by qualified dietitians (KS, WD and FL), using the food based dietary guidelines approach. All three study groups were asked to take one multivitamin tablet daily (nutrient content reflected in Table 3.2). The motivation for this low dose multivitamin tablet was the ethical issue that diagnosed HIV-positive subjects should not receive placebo capsules only. The SMMS group were also asked to take 300g of the supplement daily (nutrient content in Table 3.1), the control group would take two placebo capsules (composition in Table 3.3) from Sportron International (Cramerview, Johannesburg) and the phytochemical group would also take two capsules a day. A total of 25 subjects were recruited for the SMMS, however, by the end of the six months intervention only 16 subjects were still participating. One had died, one dropped out due to illness, two had moved to another area, and five dropped out due to unexplained reasons. Of these 16 subjects eight also participated in a social work support programme offered by a Ph.D. student/lecturer (AR) in the School for Psycho-Social Sciences at PU for CHE. This support programme entailed weekly

group sessions and was offered to all the subjects. However, only about 50% of them were willing to partake in these. The rest preferred to keep their HIV-status a secret.

Table 3.1 Nutrient composition of the soy maize meal supplement (SMMS)

Nutrient	Amount/100g	Amount/300g	Adult RDA/DRI*
Energy kJ	1790	5370	
Protein g	18 – 20	60	45 - 65
Fat g	8 – 13	39	
Carbohydrates g	64	193	
Dietary fibre g	4.9	14.7	25 - 30
Moisture g	4	12	
Lecithin g	1. 33	3. 99	
Vitamin A µgRE	121	365	700 (females) 900 (males)
Thiamin mg	0.04	0.12	1.1 (females) 1.2 (males)
Riboflavin mg	0.03	0.09	1.1(females) 1.3(males)
Niacin mg	1.35	4.	14 (females) 16 (males)
Pantothenic acid mg	0. 68		5.0
Pyridoxine mg	0. 02	0. 06	1.3
Biotin mg	0. 05	1. 56	30 µg
ISOFLAVONES MG			
Diadzein			
Genistein	177	531	
Glycetein	380	1141	
	32	96	

Analysed by SGS South Africa (PTY) Ltd, Agriculture and Food Laboratory, Christiana, South Africa for Specialized Protein Products, Potchefstroom.
*DRI = Dietary reference intakes for vitamin A, and B vitamins based on recommendations for adults aged 19 to 50 years (Food and Nutrition Board, 2001).

Table 3. 2 Nutrient composition of the multivitamin tablet

Nutrient	Amount per tablet
Vitamin A µgRE	167
Thiamin mg	0.5
Vitamin C mg	1.5
Vitamin D IU	25
Niacin mg	0.5

Produced by Portfolio Pharmaceuticals, 18 Forge Road, Spartan.

Table 3.3 Nutrient composition of the placebo capsule

Ingredient	Amount (mg)	Activity status
Sorbitol	441. 66	Non-active
Mg Stearate	4.5	Non-active
Aerosil	2. 25	Non-active
Cocoa	33. 33	Non-active (colourant/flavourant)
Total	**481. 74**	

Analysed by Biomox Pharmaceuticals (PTY) LTD, Pretoria.

3.7 Analytical methods

3.7.1 Blood sample analysis

The differential blood cells were analysed by Pathcare Laboratory in Klerksdorp using the methods and equipment indicated in Table 3.4, while the other biochemical tests were performed by the Institute of Pathology, University of Pretoria, South Africa (see Table 3.5 for methods and equipment).

3.7.2 Dietary analysis

Dietary intakes for the SMMS group were coded and analysed by PH and MM and for the antioxidants and placebo groups the data was processed by FL, KS and WD. The Food Finder programme, based on locally compiled food composition tables (Langenhoven et al., 1991) was used for analysis of the nutrients.

3.8 Statistical analysis

Further analyses to evaluate the effect of the intervention and associations between variables were performed using the STATISTICA programme by Professor Johann Jerling, School for Physiology, Nutrition and Consumer Sciences in the department (J.J). This part of the analysis was done for all the subjects who had turned up for baseline, and final evaluation sessions (see Figure 3.2). For normally distributed data parametric statistical analysis was used, and for data that was not normally distributed, log transformation calculations were performed. On the latter set of data a Shapiro-Wilk's test was done to determine normality, again data that was normally distributed was analysed using parametric statistics, and non-parametric analysis for data not normally distributed.

Table 3.4 Analytical methods and equipment for differential blood cells

Variables	Normal range	Methods & references	Place	Apparatus
Red blood cells				
Haemoglobin	13 – 18 g/dL	Photometry at 540nm	Pathcare pathology laboratory, Potchefstroom/Klerksdorp	Coulter MAX-M
Haematocrit	40 - 50 %	Calculation		
White blood cells				
Total white blood cell count	4.0 – 11.00 x 10^9 g/L	By impedance change in the orifice determined by the size of the cell.	Pathcare	Coulter MAX-M
Neutrophils	2.00 – 7.50 x 10^9 g/L		Pathcare	Coulter MAX-M
Lymphocytes	1.00 – 4.00 x 10^9 g/L 0.18 – 1 x 10^9	VCS Technology (laser)	Pathcare	Coulter MAX-M
Monocytes	g/L0.00 – 0.40 x 10^9 g/L	VCS Technology (laser)	Pathcare	Coulter MAX-M
Eosinophils	0.00 – 0.10 x 10^9 g/L	VCS Technology (laser)	Pathcare	Coulter MAX-M
Basophils		VCS Technology (laser)	Pathcare	Coulter MAX-M
Total platelet count	140 – 420 10^9/L	Impedance (see above)	Pathcare	Coulter MAX-M
Plasma viscosity	1 – 10 mm/h	By measure of pressure difference in a given length of tube with a given diameter and with a control as reference	Pathcare	Coulter Viscometer II
Total –T cells	688 – 1955 / mm^3	Flow cytometry	Pathcare	Coulter Epics
CD_4	355 – 1213 / mm^3			
CD_8	208 – 796 / mm^3			

Table 3.5 **Biochemical variables analysed by the Institute of Pathology, University of Pretoria**

Biochemical variables	Normal range	Equipment / apparatus used for analysis
Viral loads	0	Bayer ® System 340 DNA Analyser
Vitamin A	0.8 – 3.5 µmol/L	High performance liquid chromatographer (HPLC) (Hewlett Packard 1050 series)
Vitamin B12	188 – 1050 pg/mL	IMX® system
Vitamin C	0.4 – 2.0 mg/dL	HPLC and (U.V.) detection
Ferritin	20. 0 – 250 µg/L (Male) 10. 0 – 120 µg/L (Female)	Axsym® system
Alanine aminotransferase (ALT)	10 – 40 IU/L (Male) 7 – 35 IU/L (Female)	Synchron Clinical System LX20 ®
Aspartate aminotransferase (AST)	13 – 32 IU/L	Synchron Clinical System LX20 ®
Lactate dehydrogenase (LD)	90 –180 IU/L	Synchron Clinical System LX20 ®
Total protein	64 – 83 g/ L	Synchron Clinical System LX20 ®
Retinol binding protein	3. 00 – 6. 00 mg/L	Immage immunochemistry system
Albumin	34 – 48 g/L	Synchron Clinical System LX20 ®
Fibrinogen	1.8 – 3.5 g/L	Behring Fibrintimer A (BFA)
C-reactive protein (CRP)	0 – 10 mg/L	Synchron Clinical System LX20 ®
Glucose	3. 9 – 5. 8 mmol/L	Synchron Clinical System LX20 ®
Total cholesterol	3.2 - < 5.2 mmol/L	Synchron Clinical System LX20 ®
Triglycerides	0.80 – 1.50 mmol/L	Synchron Clinical System LX20 ®
High-density lipoprotein cholesterol (HDL-C)	0.9 - 2.0 mmol/L (Male) 1.0 – 2.0 mmol/L (Female)	Synchron Clinical System LX20 ®

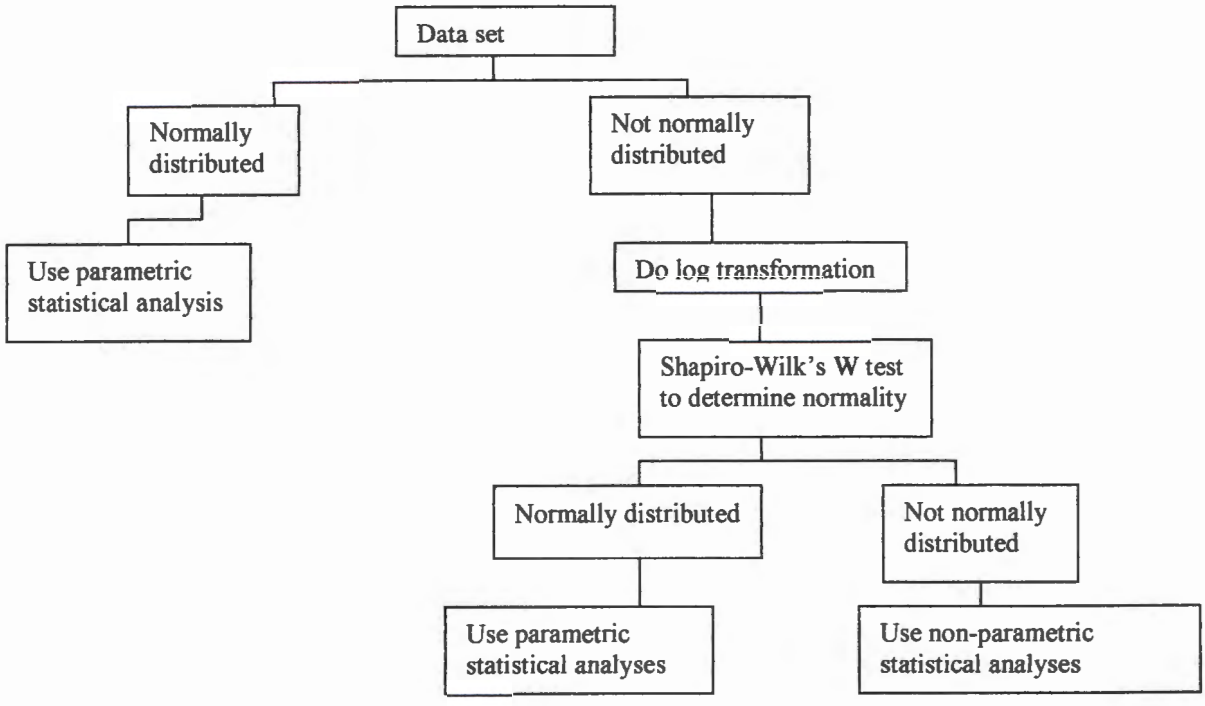


Figure 3.2 Process for statistical analysis

Differences between baseline variables and normal values were determined. The baseline variables of individual subjects were analysed for variance with the end measurements. The differences observed between baseline and end variables within each group and between the SMMS and placebo groups were also evaluated. The tests performed are described in Table 3.6 below. To perform statistical correlations the Spearman rank order were used.

Table 3.6 Tests used to assess differences between baseline and end variables within and between groups

Differences between baseline and end (within group)		**Differences in changes between groups**	
Parametric	Non-parametric	Parametric	Non-parametric
t-test for dependent samples	Wilcoxon matched pairs test	t-test for independent samples	Mann Whitney U test

3.9 Limitations/ constraints of the study

The stigmatisation and confidentiality aspect entailed in HIV/AIDS made it difficult for the patients to volunteer into the study, consequently the recruitment process was slow. The sample size was much smaller than originally planned and quite a few subjects discontinued their participation. Reasons for non-participation varied among the SMMS group. One of the subjects moved to another town, three

subjects from the farm community could not accept that they were HIV positive and consequently stopped participating. One subject found a new job, where she was not able to explain her absence from work to participate in the study. Two other subjects stopped participating due to severe illness (one of them died before completion of the study). A randomised controlled study with HIV/AIDS subjects in Bloemfontein, South Africa, found that 61.4% of the study subjects did not attend follow-up (Chikobvu et al., 2000). In our study the drop out rate was 33 %, which is lower than the Chikobvu et al. study. The reasons given for non-attendance in their study were forgetting about appointments, no reason and no longer interested. The population in the Bloemfontein study were very mobile, which made it difficult to trace the non-attenders and for them to attend follow-up visits. Some subjects in that study deliberately gave incorrect addresses and the assumption is that they wanted to maintain confidentiality of their HIV status from family members. Similar problems were encountered in our study. Similar to the study mentioned in Chapter 2 (Fawzi et al., 1998), the multivitamin tablet that was given to both study groups might have masked the effect of the intervention. The subjects were entrusted to report on compliance on ingestion of the supplements. However, there is a possibility that they shared the SMMS with other family members.

3.10 Strengths of the study

The social support and counseling components of the study gave the subjects a marked improvement in their attitudes and general quality of life, and those participating in group sessions were able to extend morale support to fellow group members in times of need, as was observed with the SMMS group. This group consisted mostly of members of the TŠepong HIV/AIDS support group. Other members of this HIV/AIDS group that did not participate in the study and the Coodinator of the group have appreciated the positive effects of the intervention (personal communication).

3.11 Summary

This study was a multidisciplinary placebo controlled intervention trial using a soy maize meal supplement (SMMS), multivitamin tablets and placebo capsules. The multivitamins were given to both study groups. At baseline 78 subjects enrolled, at the end of the six months intervention 49 subjects were remaining in the study. The drop out rate of 37% is lower than that of a study which was undertaken in Bloemfontein among HIV patients (Chikobvu et al., 2000) that experienced a dropout rate of 61.4%. The effect of the SMMS was assessed among the intervention group by comparing their dietary intakes of the

energy nutrients (fats, carbohydrates and proteins), anthropometric and biochemical variables with that of the control group. The anthropometric variables assessed included weight, BMI, and lean body mass. For the biochemical variables serum retinol level was used as an indicator for vitamin A status, the HIV infection markers included CD4 cell count, CD4 cell count as percentage of total lymphocytes and the viral load. Acute phase proteins to assess the rate of infections included RBP and C-reactive protein. In view of the small sample size the results presented in the next chapter have to be interpreted with caution.

CHAPTER 4

RESULTS

4.1 Introduction

This chapter gives a description of the findings of the study based on the objectives set in Chapter 1. The baseline health profile and demographics of the subjects in the two groups are first presented, including some characteristics of their background diets. The Kruskal-Wallis and median tests were used to determine whether any of the baseline variables differed between the two groups (non-parametric analysis of variance, ANOVA). There were no significant differences between the groups ($p>0.32$) at baseline. Therefore, the results of the interventions will be presented as changes from baseline to the end of the six month research. Comparisons were made between the changes in the two study groups using the mean and 95% confidence interval (CI) or medians and 25^{th} and 75^{th} percentiles of some variables (such as the CD4 cells) based on the distribution of the data and therefore the type of inferential statistics used (see figure 3.2). Results of selected variables are also presented for individual subjects (baseline versus end). Differences were considered significant if p was <0.05.

4.2 Description of the sample of HIV patients

The age of the study subjects ranged from 20 to 51 years with more than 70% of the subjects below the age of 35. The mean age [with standard deviations (SD)], disease classification and frequency of clinical symptoms reported are depicted in Table 4.1. The diagnosis for HIV/AIDS was based on the 1997 revision of the Centers for Disease Control (CDC) surveillance case definition (Centers for Disease Control, 1997). At baseline both groups had a higher proportion of HIV than AIDS subjects. The endline analysis showed that two subjects on SMMS, and none of the control subjects progressed into the AIDS stage during the six months of intervention, defined by CD4 cell counts less than 200 per μL (Table 4.6 to 4.9). At baseline two subjects on SMMS and three controls reported that they had been taking some multivitamin supplements, which were mainly B-complex supplied by the clinic sisters and were discontinued during the intervention. Women had a higher proportion in the study sample than men. There were 12 women and four men in the SMMS group and the control group included nine women and three men. Because of small numbers, the results will not be further divided into gender groups.

Some of the subjects reported that they were experiencing diarrhea (five), mouth sores (five) and skin rashes (four) at baseline. These complaints occurred in both the SMMS group and the control group (see Table 4.2 below). By the end of six months the occurrence of cough, diarrhoea, skin rash and mouth sores was reported by more subjects in the SMMS group than at baseline. In the control group it was only for the cough and skin rash where the number had increased as shown on the table below. The mean ear temperature as taken by the nursing sister for both groups was 37°C at baseline. It stayed the same for the SMMS group, and slightly dropped to 36° C for the controls.

Table 4.1 **Baseline and end demographic (age) and health characteristics (frequency of symptoms reported or observed) of HIV positive subjects per study group**

Characteristics	**SMMS n =16**		**Control n = 12**	
Mean age (SD) in years	Total group 28 (± 5)		Total group 31 (± 8)	
	Women 27 (±3) Men 30 (±6)		Women 29 (±7) Men 40 (±10)	
HIV/AIDS classification	2 AIDS/14 HIV		0 AIDS/12 HIV	
	Baseline	End	Baseline	End
Cough (n)	1	3	1	3
Diarrhea (n)	2	3	3	1
Nausea (n)	0	0	0	0
Average temperature°C	37	37	37	36
Abdominal cramps (n)	0	0	0	0
Skin rash (n)	1	3	3	4
Mouth sores (n)	3	5	2	0

(n) = number/s

4.3 Dietary intakes

The ratio of energy intake (EI) to basal metabolic rate (BMR) was calculated as a guide for the accuracy of the dietary records (both the 24 hour recall and the QFFQ). An EI:BMR below 1.2 is regarded as an energy intake too low for the maintenance of body weight (Goldberg et al., 1991). Some subjects scored below 1.2 on the 24hour recall, and were considered as under reporters (Appendix H). Consequently only the results of the QFFQ will be reported in this mini-dissertation.

For the dietary intakes, this study will only report on the macronutrients (carbohydrates, fats and proteins), energy and vitamin A, even though full analysis of other nutrients was made. The baseline and endline dietary intakes for total energy, carbohydrates, vitamin A, fat and protein by type of intervention are shown in Table 4.2. Comparisons of the mean intakes of the two groups were made. Analysis of the dietary intake revealed that the mean intake of energy, macronutrients and vitamin A by the two study groups had decreased by the end of the study period of six months. There was no significant difference in changes for dietary intakes between the two study groups.

Comparison of baseline dietary intakes for study subjects on SMMS (without the supplement) and controls (Table 4.2) showed that the latter group had lower intakes for all nutrients analysed (total protein, fat, carbohydrate and vitamin A). Both groups consumed less than 30% of their energy as fat, and carbohydrate intakes were more than 55% of energy. Protein intake was 163% and 140% of the US RDA (Whitney et al., 1998) for the SMMS and control groups respectively. Vitamin A intake was 133% and 77% of the dietary reference intakes [(DRIs), Food and Nutrition Board, 2001] for the respective groups.

The endline intakes of fat, carbohydrate and vitamin A were lower (non-significant) than those reported at baseline for the two study groups as shown in Table 4.2 below. Protein intake for the intervention group increased but decreased (not significant) for the controls groups. Energy from fat intakes by the two study groups were still below the recommended 30% maximum, while the mean energy intake from carbohydrate was above 55% for both study groups.

4.3.1 Energy

At baseline the mean energy intake of the SMMS group was 11986 kJ (95% CI: 9824 - 14624) and the control group's intake 10919 kJ (95% CI: 8653 – 13779). These mean intakes were slightly higher than the US RDA for men and women aged 10 to 50 at a mean of 10659 kJ (Whitney et al. 1998). After six months the energy intake for the SMMS group excluding the supplement, had significantly decreased ($p = 0.01$) to 8816 kJ (95% CI: 7011 – 11086). The mean energy intake for the controls tended to decrease to 9150 (95% CI: 6917 – 12103) constituting 86% of the RDA. However, the SMMS group reported an additional mean daily intake of 5370 kJ from the supplement during the six months of intervention, which significantly increased the mean daily intake to 14 186 kJ and raised the energy intake to 133 % of the RDA.

Table 4.2 Comparison of baseline and end dietary variables of SMMS (without and with supplement) and control groups

	SMMS Baseline n = 16		SMMS End n = 15	SMMS End with supplement		t-test	Controls Baseline n = 15		Controls End n = 12		Change	t-test
Variables	Mean & 95 % CI	%RDA/DRIs	Mean & 95 % CI	Total	% RDA/DRIs	p	Mean & 95 % CI	% RDA	Mean & 95 % CI	% RDA/DRIs		p
Total energy (kJ)	11986 (9824 - 14624)	112	8816 (7011 – 11086)	14186 (12381 – 16456)	133		10919 (8653 – 13779)	103	9150 (6917– 12103)	86	-1769	0.60
Fat (g)	62 (49 – 79)	95	53 (43 – 63)	77 (82 – 102)	118		52 (43 – 62)	80	49(28 – 69)	75	-3	0.34
% of energy from fat	19	NA	23	21	NA		18	NA	20	NA	2	ND
Carbohydrate (g)	483 (394-594)	161	366 (263 – 511)	559 (456 - 704)	186		448 (338 – 593)	149	367 (281 – 479)	122	-81	0.65
% of energy from carbohydrate	67	NA	69	66	NA		69	NA	67	NA	2	ND
Protein (g)	92 (69 – 104)	142	72 (40 – 103)	126 (94 – 163)	195		79 (63 – 99)	144	57 (47 – 68)	100	-22	0.06
% of energy from protein	14	NA	18	15	NA		13	NA	10	NA	-9	
Vitamin A (μgRE)	1058 (385 – 1841)	132	584 (459 – 709)	*1116 (1045 – 1241)	140		1396 (62 – 2730)	175	617 (129 – 1105)	77	- 779	0.39

* Including vitamin A from multivitamin tablet.
RDA = US RDA (Whitney et al. 1998).
DRIs = Dietary Reference Intakes (Food and Nutrition Board, 2001).
ND = Not determined.
NA = Not applicable

4.3.2 Vitamin A

The mean baseline vitamin A intake of the SMMS group was 1058 µgRE (95% CI:385-1841), which was within the limits of the dietary reference intakes (DRIs) of 700 to 900µgRE for women and men respectively, as recommended by the US Food and Nutrition Board (FNB) in 1998 -2001 (Food and Nutrition Board, 2001). The control group had a slightly higher mean intake of 1396µgRE (95% CI: 62 – 2730). By the time the endline measurements were taken the group on SMMS consumed an additional daily mean intake of 315µgRE from the supplement and multivitamin tablet. The control group was consuming an additional 151µgRE from the multivitamin supplement. The mean total daily intake of the control group by the end of the six months study was therefore 768µgRE. None of these changes were statistically significant.

4.3.3 Total protein

The group on SMMS reported comparatively higher total protein intakes (92g) than the control group (79g) at baseline. Both groups reported intakes higher than the RDA for adults of 50g for women and 63g for men (FNB, 1989). At the end of six months the SMMS group was consuming relatively more total protein from the diet than the control group ($p = 0.57$). The end mean intake values for the SMMS group was above the RDA at 152g. The endline mean daily protein intake for the control subjects was 57g, which was significantly lower than the baseline measurements ($p = 0.01$). This level was also lower than the RDA.

4.4 Anthropometry

HIV/AIDS patients usually lose weight, body fat and lean body mass with disease progression (Dowling et al., 1990). In this report the anthropometric results that will be given are on weight, BMI and lean body mass. There were no significant differences between the SMMS and control groups on baseline anthropometric measurements ($p = 0.50$ for weight, $p = 0.41$ for BMI and $p= 0.13$ for lean body mass) and for the decrease or increase incurred within groups during the study (see Table 4.3). The end mean BMI of both study groups were within the acceptable range of 20 to 25 (Whitney et al., 1998). However, four subjects on SMMS and three controls had a BMI below 20 at baseline and after six months

Table 4.3 **Comparison of means and 95% confidence intervals of baseline and end anthropometric variables in groups**

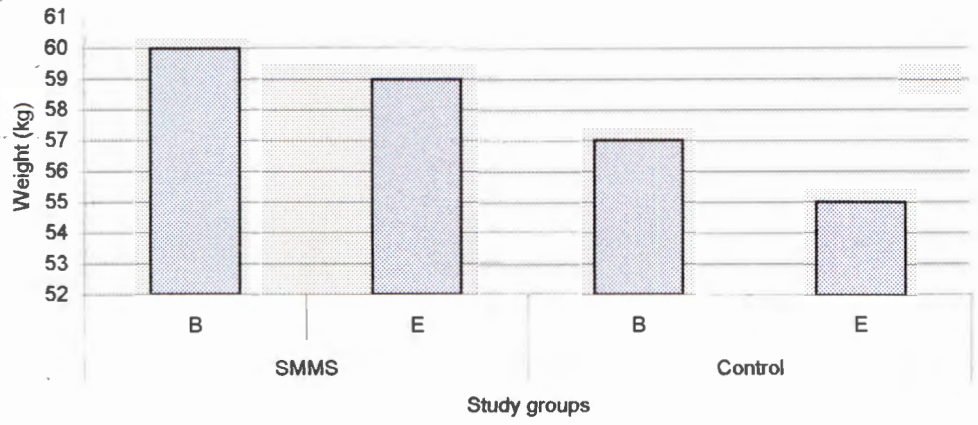
	SMMS Baseline n = 16	**SMMS End n = 15**	**Change**	**t-test**	**Controls Baseline n = 15**	**Controls End n = 12**	**Change**	**t-test**
Variables	Mean (95 % CI)	Mean (95 % CI)		p	Mean (95 % CI)	Mean (95 % CI)		p
Body weight (kg)	60 (55 – 65)	59 (53 – 65)	-1	0.65	57 (51 – 63)	55 (48 – 61)	- 2	0.11
BMI (kg/m^2)	23 (21 – 25)	22 (21- 24)	-1	0.66	23 (21 – 24)	22 (21 – 24)	- 1	0.92
Lean body mass (kg)	45 (42 – 49)	45 (41 – 50)	0	0.63	42 (39 – 46)	42 (37- 47)	0	0.63

No significant differences between baseline and end, or between changes in groups.

4.4.1 Weight, BMI and lean body mass

The baseline and end mean body weight of the SMMS group were somewhat higher than the control group (not significant). The mean BMI at baseline for both groups were within the acceptable category for both men and women (20 –25 kg/m^2, Whitney et al., 1998). The mean weight of the SMMS group decreased by one and the controls by two kilograms over the six months period of intervention. There were no significant differences between the baseline and end measurements within the groups. Figure 4.1 below illustrates the mean weights of the two study groups, measured at baseline and after six months. The mean lean body mass for both groups stayed almost the same over the six months period (Table 4.3).

Analysis of the trend of body weight changes for individuals is reflected in Figures 4.2 and 4.3. The body weight of 10 subjects on SMMS increased during the six months, three of them gained weight during the first three months and maintained it at the same level for the next three months. Two others among the ten with weight gain and one who maintained the same weight over six months, experienced a drop in weight after the first three months, but regained it.

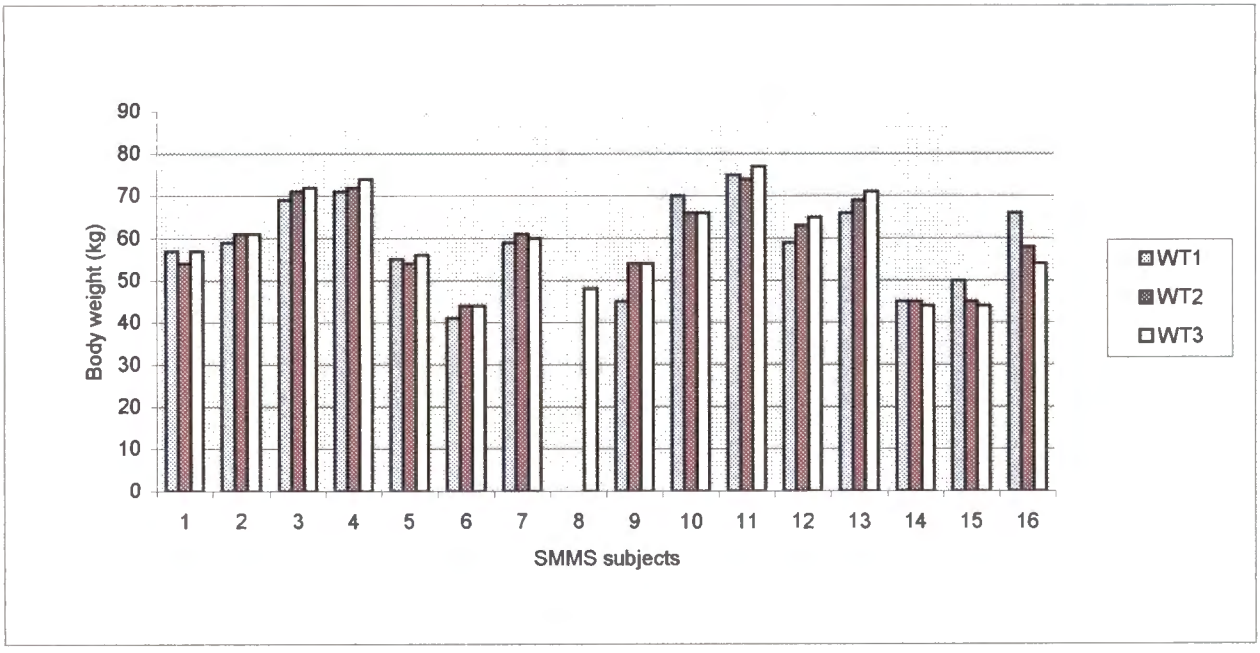


B = baseline E = end

Figure 4.1 Mean body weight of study groups at baseline and end

Four subjects had a decrease in weight during the study period. Among them three lost weight during the first three months and over the last three months their weight remained almost constant, with the exception of one who lost 4 kilograms (subject 16 in Figure 4.2).

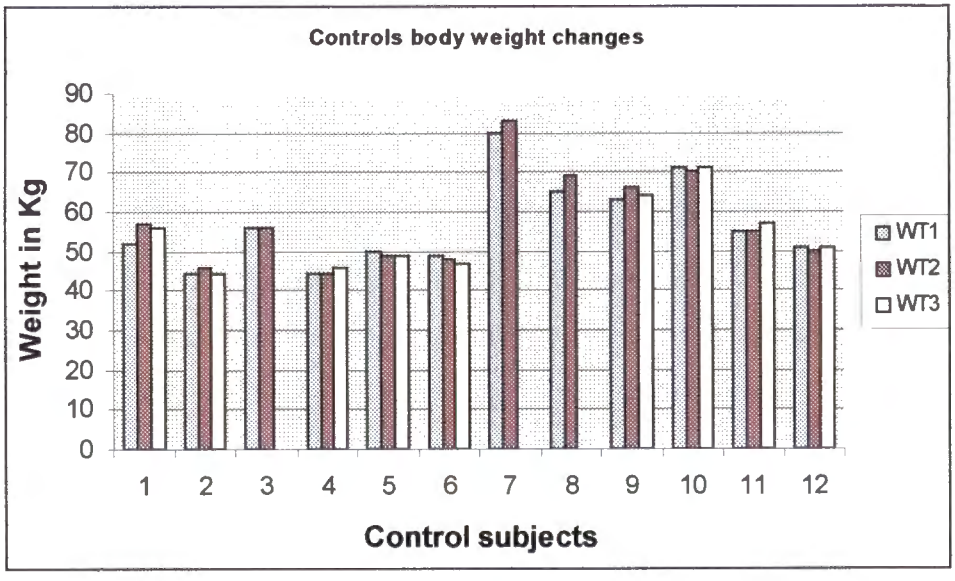
Analysis for trends of body weight changes of the control group (Figure 4.3) showed that the weight of six subjects increased over the six months. Two of them had a constant weight during the first three months of the study, which increased over the remaining three months, while two others experienced an increase during the first three months and a slightly decrease at the final assessment. Midline measurements of two subjects with weight increase were not available. Only end weight was measured for subject number 8 in the SMMS group. Two control subjects with constant body weight at baseline and end experienced a rise over the first three months, but the weight gained had decreased by the end of the remaining six months. Two other subjects with similar weight at baseline and end experienced a decrease during the first three months which was regained during the remaining later months.



WT 1 = baseline body weight , WT2 = body weight after three months, WT3 = body weight after six months

Figure 4.2 Body weight changes in the SMMS group

Only two control subjects lost weight. One of these (subject five in Figure 4.3) lost about 600g over three months and maintained a constant weight over the remaining months of the study, while one had a steady decline (Subject Six in figure 4.3).



WT 1 = baseline body weight , WT2 = body weight after 3 months, WT3 = body weight after 6 months

Figure 4.3 Body weight changes in the control group

4.5 Biochemical changes

Comparisons on the changes in levels of CD4 count, RBP and serum retinol among the study subjects (see Table 4.4) show that the SMMS group experienced a significant mean decrease in CD4 count of -82 at p = 0.003, and RBP of 0.9 (3.6 to 2.7 mg/L, p = 0.02) and a non-significant decrease in serum retinol of 0.3μmol/L. The control group experienced the least non-significant decrease in serum retinol at 0.04μmol/L, RBP at –0.5 mg/L and CD4 cell count of –26 cells/μL.

4.5.1 Serum retinol levels

The serum retinol levels of all the study subjects were either within or higher than the normal range suggested by the IVACG (0.7 – 1.05μmol/L, Underwood & Olson, 1993) at baseline and end. The cut-off point for normal values of vitamin A used by the University of Pretoria pathology laboratory is 0.8 to 3.5μmol/L. Among the group on SMMS, 10 subjects experienced a decrease in serum vitamin A ranging from 0.1 to 1.4 μmol/L during the study while four had an increase from the baseline ranging from 0.2 to 0.9 μmol/L, and the levels for two subjects remained the same (Figure 4.4).

Among the 12 controls the serum retinol levels for two subjects remained the same as at baseline, five had increased and five had decreased levels after six months (Figure 4.5). The increase occurred only among subjects with a CD4 count between 200 and 500, while the decrease included subjects with and those above 500 as shown in Tables 4.5 and 4.6.

Table 4.4 **Comparison of means and medians of baseline and end biochemical variables in and between groups**

Variables	SMMS Baseline n = 16	SMMS End n = 15	Change	t-test	Controls Baseline n = 15	Controls End n = 12	Change	t-test
	Mean (95 % CI)	Mean (95 % CI)		p	Mean (95 % CI)	Mean (95 % CI)		p
RBP (mg/L)	3.6 (3 – 4.3)	2.7 (2 – 3.4)	-0.9 (±1.4)	0.02	3.5 (2.6 – 4.4)	3 (1.8 – 4.3)	-0.5 (±1.6)	0.34
Retinol (µmol/L)	2.0 (1.7 – 2.3)	1.7 (1.4 – 2.0)	-1.2 (±1.4)	0.91	1.8 (1.4 – 2.0)	1.8(1.4 –2.3)	-0.04 (±0.1)	0.913
CD4 as % total lymphocyte	24 (18 – 33)	2.5 (20 – 31)	1	0.84	25 (21– 2.9)	28 (23 – 34)	3	0.13
CD4:CD8	0.4 (0.27 – .5)	0.4 (0.3 – 0.5)	0	0.41	0.4 (0.3 – 0.5)	0.5 (0.4 – 0.6)	0.1	0.88
Viral load (copies/mL)	105800 (3653 – 207946)	116886 (37229 – 196544)	11176 (±112569)	0.71	50253 (-4783 – 105288)	47656 (15243 – 80068)	-2597 (±56520)	0.88
	Median (25th and 75th percentiles)	Median (25th and 75th percentiles)	Change	p	Median (25th and 75th percentiles)	Median (25th and 75th percentiles)	Change	p
CD4 (cells/mm3)	351 (242 – 644)	268 (182 – 489)	-83	0.003	384 (341 – 503)	358 (313 – 438)	-26	0.21

RBP = Retinol binding protein

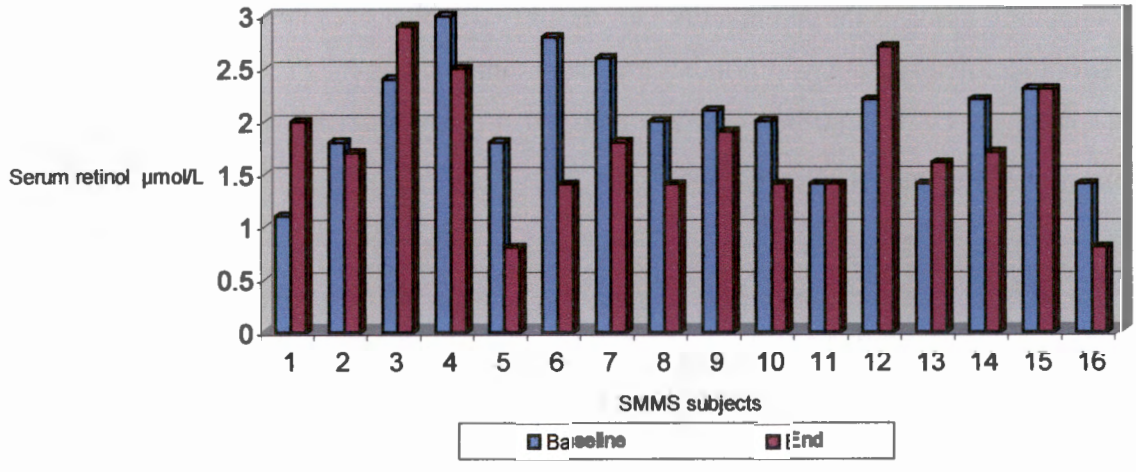


Figure 4.4 Individual serum retinol values at baseline and end for SMMS subjects

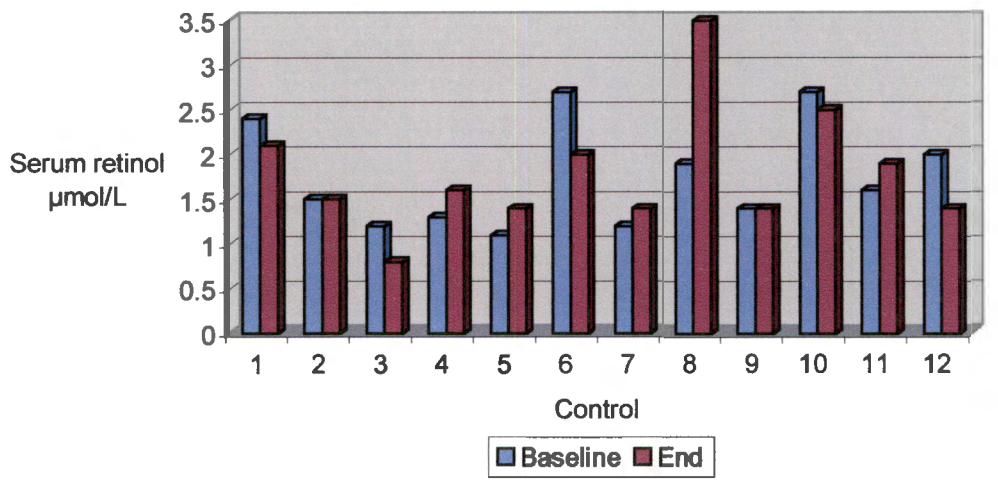


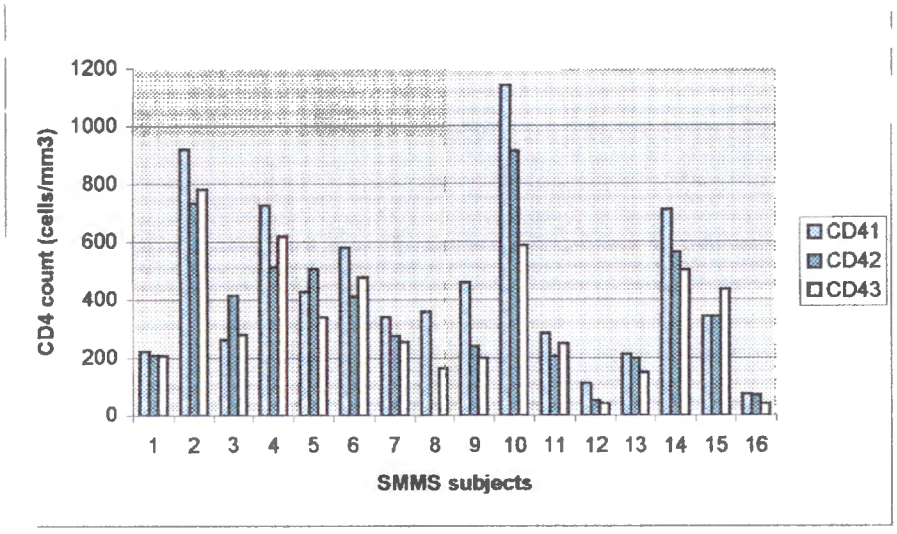
Figure 4.5 Individual serum retinol values at baseline and end for controls

4.5.2 CD4 counts

Figure 4.6 shows the trend of CD4 counts for SMMS subjects at baseline, after three months (midline) and six months (end). Eight subjects had a steady decline in CD4 count at midline and end. Four experienced a decrease after three months and an increase in the following three months. Two subjects had an increase in CD4 count during the first three months of the study and an increase after the last three months. One other subject had similar levels of CD4 count at baseline and midline, the final assessment the levels had increased to over 400 cells/mm^3 similar levels of CD4 count at baseline and midline, the final assessment the levels had increased to over 400 cells/mm^3.

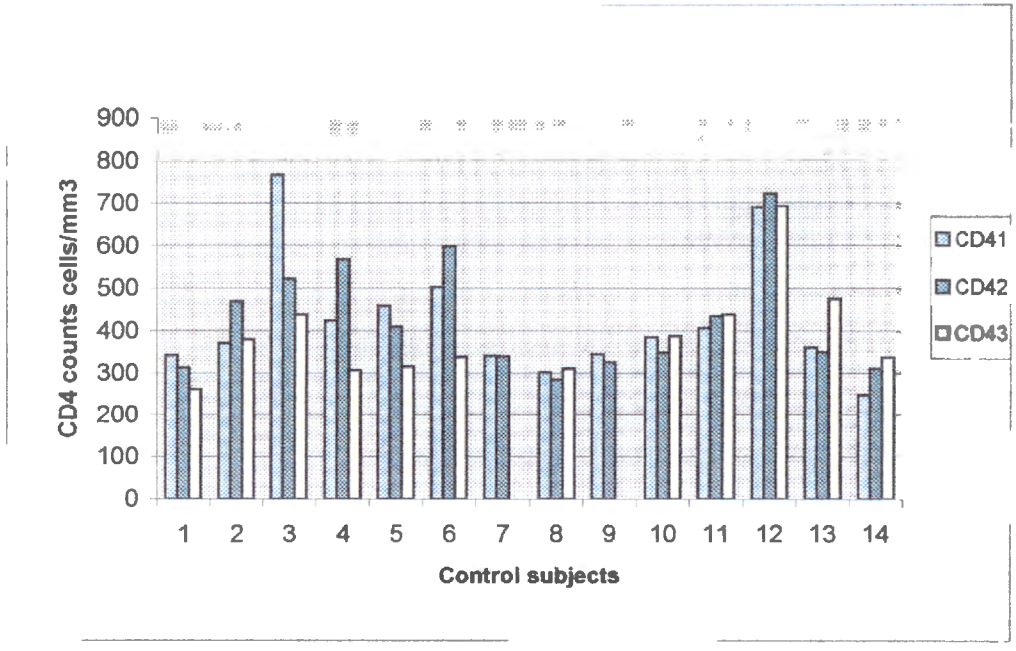
The trend of CD4 count increase among the control subjects is reflected in Figure 4.7. Five control subjects experienced a decrease in CD4 count. Among these the cell count of the two subjects had increased during the midline assessment, and then decreased during the second half of the study. Among the four subjects with counts that remained constant, the CD4 count of two controls had increased after the first three months of the study, but decreased at the final assessment. One of these four subjects had a decrease during the midline assessment. One of the three subjects with an increase in CD4 count experienced a decrease after the three months and an increase at the final assessment. Two subjects did not have end CD4 counts. From the results depicted in Figures 4.6 and

4.7 it is clear that CD4 counts fluctuated in most of the subjects, which corresponds with



the information supplied by the New Mexico AIDS Infonet (1997).

Figure 4.6. Individual CD4 counts at baseline, midline and end for SMMS subjects
CD41 = baseline CD4 count, CD42 = midline CD4 count, CD43 = end CD_4 count



CD41 = baseline CD4 count, CD42 = midline CD4 count, CD43 = end CD4 count

Figure 4.7 Individual CD4 counts at baseline, midline and end for control subjects.

4.5.3 Serum retinol and CD4 count

Serum retinol levels stratified by CD4 cell count showed that higher levels of the vitamin were found with an increase in CD4 cell count as depicted in Table 4.5. Even after the six months period subjects with a CD4 count above 500 cells/ mm^3 had higher serum retinol levels. These trends occurred among both subjects groups. Most of the study population were within the group with CD4 cell count below 500 (12 SMMS subjects and 11 controls at the end).

Table 4.5 Mean retinol level in µmol/L stratified by CD4 cell count and type of intervention

			CD4-cell count		
Study group	n		≤ 200 (n)	≤ 500 (n)	> 500 (n)
SMMS Serum retinol µmol/L	16	Baseline	1.80 ± .57 (2)	1.90 ± 51 (9)	2.36 ± .52 (5)
		End	1.68 ± .70 (5)	1.80 ± 69 (7)	1.83 ± .47 (4)
Controls Serum retinol µmol/L	12	Baseline		1.60 ± 0.42 (9)	2.20 ± 0.87 (3)
		End		1.73 ± 0.69 (11)	2.50 (1)

The results in Table 4.6 and 4.7 show that four subjects with higher CD4 levels (≥500 cells/ mm^3) also had baseline serum retinol levels higher than 2µmol/L. However, after six months a decline occurred in both variables among these subjects, although none reached the low serum retinol margin. Neither did the CD4 count decrease to lower than 500, except for one subject, whose end CD4 count decreased to 477. Among the nine SMMS subjects with a CD4 count between 200 and 500, two experienced a total CD4 increase of 17 and 93. The decrease of CD4 cell count in this sub-group ranged from 14 to 258 cells/ mm^3 over the six months, where two progressed into the AIDS stage of CD4 cells less than 200. The baseline retinol levels were adequate according to IVACG standards, ranging from 1.1 to 2.6 µmol/L. Four subjects had a decrease in serum retinol, though they were within the adequate level.

Two subjects on SMMS with a CD4 count less than 200cells/mm^3 experienced a reduction in CD4 count during the study period of six months. Both subjects had baseline serum retinol levels above the 1.05µmol/L cut-off point for adequate levels. One of them also experienced an increase in retinol.

Table 4.6 Serum retinol levels of individual SMMS subjects stratified by CD4 cell counts

Baseline CD4 cells/ mm^3	End CD4 cells/ mm^3	Change	Baseline retinol µmol/L	End retinol µmol/L	Change
Serum retinol levels of subjects with CD4 counts less than 200 cells/mm^3					
113	42	-71	2.2	2.7	0.5
76	41	-35	1.4	.8	-0.6
Serum retinol levels of subjects with CD4 counts of 200 to <500 cells/mm^3					
213	150	-63	1.4	1.6	0.2
359	169	-190	2	1.4	-0.6
221	207	-14	1.1	2	0.8
263	280	17	2.4	2.9	0.5
428	341	-80	1.8	.8	-1
340	256	-84	2.6	1.8	-0.8
458	200	-258	2.1	1.9	-0.2
284	250	-34	1.4	1.4	0
342	435	93	2.3	2.3	0
Serum retinol levels of subjects with CD_4 counts > 500 cells/ mm^3					
734	617	-87	3	2.5	-0.5
579	477	-102	2.8	1.4	-1.4
1139	586	-553	2	1.4	-0.6
708	501	-207	2.2	1.7	-0.5
921	783	-138	1.8	1.7	-0.1

The control subjects with baseline CD4 counts over 500 all had a decrease in both CD4 cell count and serum retinol ranging from 0.4 to 0.7 µmol/L for the latter variable (Table 4.7). None of these subjects progressed to the AIDS stage, though one had a serum retinol level of 0.8 µmol/L, which falls below the adequate cut-off point. None of the control subjects with baseline CD4 cell counts between 200 and 500cells/ mm^3 progressed into the AIDS stage. Three experienced reductions ranging from –63 to -143, and six controls had an increase of 2 to 115 CD4 cells/ mm^3.

Table 4.7 Serum retinol level of individual control subjects stratified by CD4 cell counts

Baseline CD4 cells/mm^3	End CD4 cells/ mm^3	Change	Baseline retinol µmol/L	End retinol µmol/L	Total Change
Serum retinol levels of subjects with CD4 counts of 200 to <500 cells/mm^3					
284	337	53	2	1.4	-0.6
360	475	115	1.6	1.9	0.3
406	438	32	1.4	1.4	0
384	386	2	1.9	3.5	1.6
302	310	8	1.2	1.4	0.2
459	316	-143	1.1	1.4	0.3
424	307	-117	1.3	1.6	0.3
369	378	9	1.5	1.5	0
324	261	-63	2.4	2.1	-.3
Serum retinol levels of subjects with CD4 ≥500 cells/mm^3 at baseline					
503	338	-165	2.7	2	-0.7
689	619	-70	2.7	2.5	-0.2
766	437	-329	1.2	1.2	-0.4

4.5.4 Serum retinol binding protein (RBP)

RBP is a negative acute-phase protein. Therefore a low level could result from protein-energy malnutrition or inflammatory stress. A significant decrease was observed from the mean baseline to the mean end RBP concentration of the SMMS group, from 3.6 to 2.7mg/L ($p = 0.02$). The decrease in the control group was not statistically significant from 3.5 to 3.0 mg/L RBP. The RBP of 14 subjects on SMMS decreased except for two subjects. An increase in both dietary and serum vitamin A was observed for one of these. The other subject reported a decrease in dietary vitamin A, but had an increase in serum vitamin A. Another three had an increase in both dietary and serum vitamin A levels, but experienced a slight decrease in RBP. The pattern was similar with the placebo group, where three out of the 12 subjects had an increase in dietary and serum vitamin A and RBP.

4.5.5 Viral load, CD4 count and serum retinol level.

Tables 4.8 and 4.9 show the viral load of individual study subjects at baseline and end. Out of the 28 study subjects three showed an increase in CD4 count and a decrease in viral load with either an increase in serum retinol or no difference in baseline and end levels. All these subjects were control subjects. One subject in the SMMS group had a decline in viral load and an increase in serum retinol.

Table 4.8 **Individual baseline (B) and end (E) values for viral load, serum retinol and CD4 count among SMMS subjects**

Subject number	Viral load (B)	Viral load(E)	Change	CD4 (B)	CD4 (E)	Change	Retinol (B) µmol/l	Retinol (E) µmol/l	Change
1	67022	130018	62996	221	207	-14	1.1	2	0.9
18	198	838	640	921	783	-138	1.8	1.7	-0.1
20	6338	9634	3296	263	280	17	2.4	2.9	0.5
22	83106	87580	4474	734	617	-117	3	2.5	-0.5
24	ND*	115540		428	341	-87	1.8	.8	-1.0
25	602378	270590	-331788	579	477	-102	2.8	1.4	-1.4
27	41590	42792	1202	340	256	-84	2.6	1.8	-0.8
72	53730	240420	186690	359	164	-195	2	1.4	-0.6
74	18360	145372	127012	458	200	- 258	2.1	1.9	-0.2
76	556	1348	792	1139	586	-553	2	1.4	-0.6
83	60676	128294	6761	284	250	-34	1.4	1.4	0
85	75266	91740	16474	113	42	-71	2.2	2.7	0.5
86	75004	20700	- 54304	213	150	-63	1.4	1.6	0.2
102	1388	1636	248	708	501	-207	2.2	1.7	-0.5
103	1384	3606	2222	342	435	93	2.3	2.3	0
104	500000	580070	80070	76	41	-35	1.4	.8	-0.6

ND* = Not determined

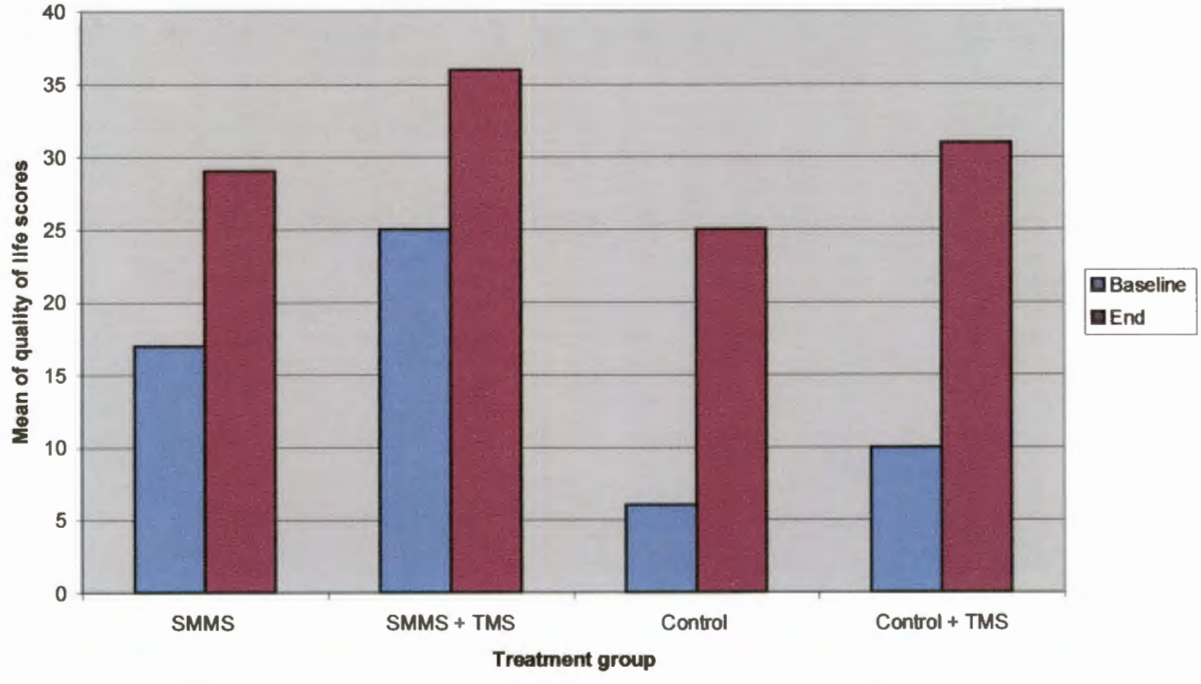
By the end of the six months intervention period, six controls had an increase in CD4 cell count, ranging from nine to 115. All of them could be classified as HIV patients, because they had a CD4 cell count above 200 cells/mm^3. The mean CD4 count for the control group after six months was 384 cells/mm^3. Three of these subjects had an increase in serum retinol (see Table 4.9). Out of the six subjects with an increase in CD4 count, four also had an increase in weight, and two of them an increase in lean body weight. One of the six only experienced an increase in lean body weight.

Table 4.9 **Individual baseline and end values for viral load, serum retinol and CD4 count among control subjects**

Subject number	Viral load (B)	Viral load(E)	Change	CD4 (B)	CD4 (E)	Change	Retinol (B) µmol/l	Retinol (E) µmol/l	Change
12	300138	127836	-172302	324	261	-63	2.4	2.1	-0.3
13	111080	121466	20386	369	378	9	1.5	1.5	0
14	19876	80072	60196	766	437	-329	1.2	.8	-0.4
17	106	326	220	424	307	-117	1.3	1.6	0.3
35	12582	24756	12174	459	316	-143	1.1	1.4	0.3
40	164	1066	902	503	338	-165	2.7	2	-0.7
51	50816	10467	-40349	302	310	8	1.2	1.4	0.2
58	82550	113654	31104	384	386	2	1.9	3.5	1.6
60	1068	732	-336	406	438	32	1.4	1.4	0
64	310	390	80	689	619	-70	2.7	2.5	-0.2
95	1048	3372	2324	360	475	115	1.6	1.9	0.3
100	49280	47380	-1900	284	337	53	2	1.4	-0.6

4.6 Quality of life

Figure 4.8 shows the results obtained on quality of life scores. There was an improvement in the experimental and control groups (with or without social support). However, analysis of data for individual study subjects indicate that improvement was not detected for one subject on SMMP and treatment for social support (TMS), and another on SMMP only and one control. The improvement observed in the control group from baseline to end was statistically highly significant ($p = 0.0005$). However, the SMMS TMS support group ended with a higher score than any of the control groups. The SMMS groups had started with higher scores for quality of life, and the room for improvement was limited.



TMS = Treatment for social support

Figure 4.8 Results on quality of life

4.7 Statistical correlations

Comparison of dietary and serum vitamin A levels on a scatter plot tended to point to negative correlation between these two variables amongst the subjects on SMMP, where the points were scattered along a slope going in the negative direction as shown on Figure 4.9. For the control group the points were also scattered in the similar direction (Figure

4.10). The more closely the points would be packed along a diagonal slope in the positive direction the higher the correlation would be considered (Polit & Hungler, 1987).

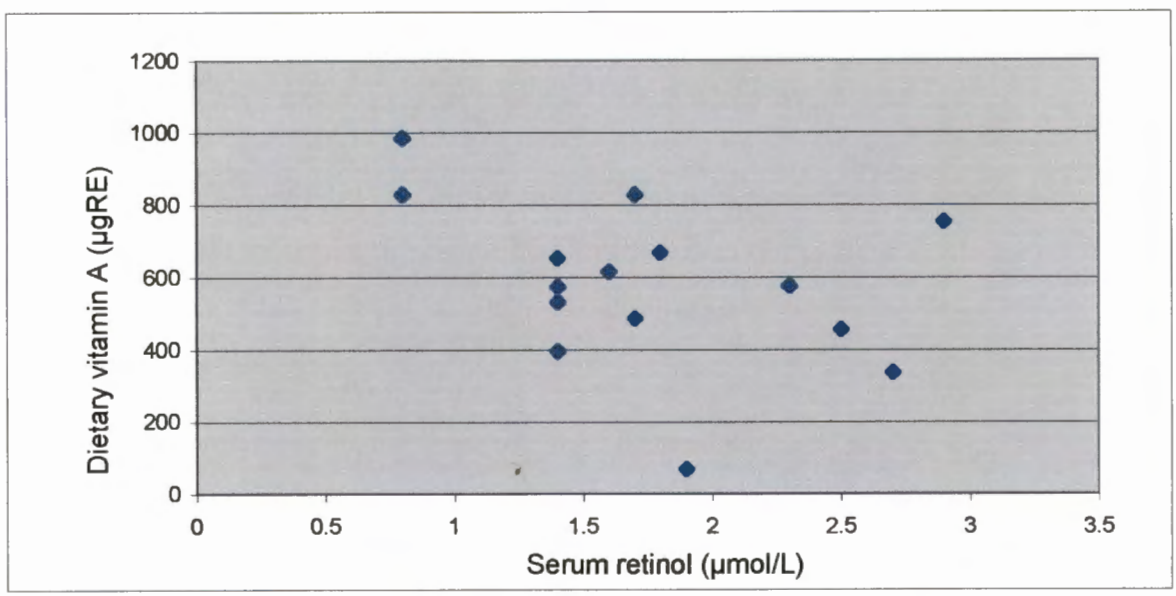


Figure 4.9 Scatter plot of dietary and serum retinol end values of the SMMP group

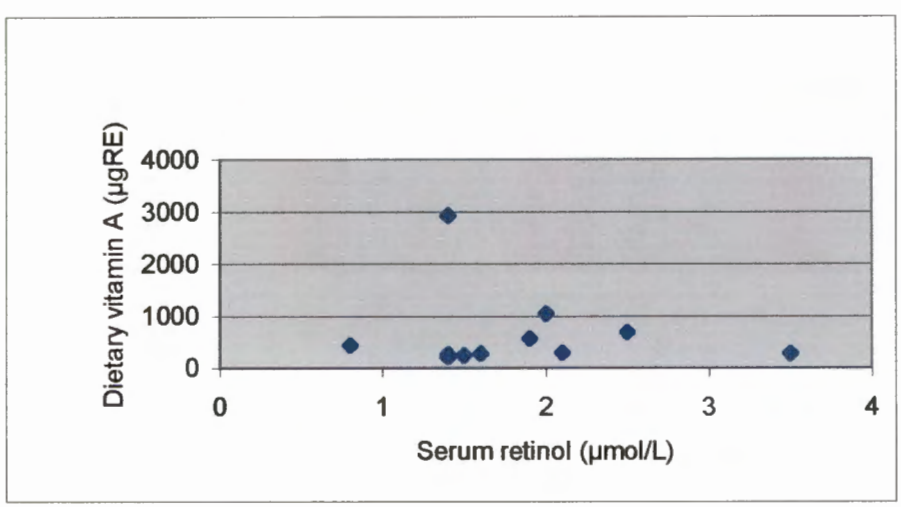


Figure 4.10 Scatter plot of dietary vitamin A and serum retinol end values of the control group

Further statistical analysis using Spearman rank correlations did not show any association between serum retinol and CD4 count below 200 cells/mm^3 ($p = 0.33$ at baseline and 0.69

at end). Neither did an association exist with a CD4 count above 500 at $p = 0.09$ and below 500cells/ mm^3 at $p = 0.50$ for baseline. Comparison of dietary vitamin A intakes and serum retinol at baseline and end (both groups combined) did not show an association. However, there was an association between RBP and serum retinol ($r = 0.81$, $p< 0.01$) and C-reactive protein and serum retinol ($r = -0.27$, $p = 0.06$) at baseline and end.

The sample size in this study was not large enough, therefore the results obtained should be interpreted with caution. This study established that the nutrient intakes from the diet of the subjects had declined by the end of the six months. The total nutrient intake of the SMMS group was increased by the supplement, relative to that of the controls, who only ingested the multivitamin in addition to the diet. The anthropometric measurements of both the intervention and control groups remained the same over six months. The markers for HIV infection progression such as CD4 count and CD4 as % total lymphocyte showed a decrease in the SMMS group at $p < 0.01$ for the former variable and $p = 0.05$ for the later. RBP also decreased significantly in this group. Serum retinol levels were within the adequate levels for both groups at baseline and end, though these levels tended to decline in individuals with a decrease in CD4 count.

Further analysis to explore the association between variables that would establish the significance of the intervention (SMMS) on delay of progression of the HIV infection, showed that there was an association between acute phase proteins and serum retinol level. The occurrence of infections such as cough, mouth sores, skin rash and diarrhoea were reported more often during the final assessment relative to baseline. At the end there were more subjects with infections among the SMMS than in the control group. The next chapters will relate to these results, comparing and contrasting with findings of other studies. Conclusions and recommendations will therefore be based on the results reflected within this section.

CHAPTER 5

DISCUSSION

5.1 Introduction

The rate of HIV progression can be assessed by the CD4 decline and compromised nutritional status, measured by biochemical and anthropometric indicators (Peakman & Vergani, 1997). These authors also mention that with progression of the infection the viral load in the host will increase and the CD4 T-lymphocyte cell that the virus replicates in will be simultaneously reduced. The variation between values for almost all variables in this study was too wide for substantial conclusions to be made, therefore this chapter will explore cautiously the possibility of a relationship between the rate of CD4 decline to levels of serum retinol and dietary vitamin A.

5.2 Dietary intakes

The dietary nutrient intakes for the control group decreased over the six months period, while that of the SMMS group increased. The limitations of the QFFQ are acknowledged. However, this decrease which occurred in the control group is expected in HIV/ AIDS subjects with disease progression (Friis & Michaelsen, 1998; McCorkindale et al., 1990). The decrease in dietary intakes may be due to the increasing rate of infections, anorexia and other factors associated with the progression of HIV infection mentioned in Chapter 2 on literature review (Augustus, 1998; Smith et al., 1992). In this study the occurrence of infections was reported somewhat more frequently by the group on SMMS (See Table 4.1). It can be assumed that this frequency of infections would require an increase in the utilization of vitamin A (Jolly et al., 1997; Lacey et al., 1996; Semba et al., 1996), hence the SMMS group experienced a greater non-significant decrease in the mean serum retinol at –1.2 μmol/L relative to –0.04μmol/L for the control group. The decrease in serum RBP was greater in the SMMS group at –0.9mg/L ($p=0.02$) relative to the controls at -0.5 mg/L ($p=0.34$), which is also an indication of infections.

The SMMS provided additional nutrients, and it was expected that the nutritional status of subjects in this group would be better than that of the control group. The energy intake could have had a direct contribution to the maintenance of body weight and BMI of the SMMS subjects, who only lost 1kg relative to 2 kg lost by the control group ($p>0.05$). A longitudinal intervention study was undertaken by Dowling et al. (1990) among 34 HIV/AIDS subjects in Dublin, Ireland. Their subjects were put on a prescription of supplements with a combination of Sanatogen multivitamins and iron and food allowance from the Department of Social Welfare. Their subjects experienced an increase in body weight, BMI and percentage body fat after 12 weeks of the intervention. This increase in anthropometric variables was attributed to the increase in nutrient intake. Another study in Califonia by McCorkindale et al. (1990) found a significant decrease ($p<0.05$) in weight, percentage body fat and BMI over a period of 16 months among HIV/AIDS study subjects, even though the food records indicated adequate nutrient intakes with the exception of total energy, vitamin B6 and zinc, similar to findings in this study where the energy intake of controls decreased over the six months period. In Africa, a cross sectional descriptive study was conducted in Abidjan (Castetbon et al., 1997). Subjects with CD4 counts below 200 cells/mm^3 had significantly lower mean weight, arm circumference and muscular circumference, than those with higher cell counts. The energy intakes of these subjects were lower than the WHO recommended intakes. These observations showed that in HIV patients other clinical infections would cause a reduction in nutrient intakes, consequently the anthropometric measurements are lowered with disease progression. However, in situations where the dietary energy intakes are higher than the recommended allowances, the ideal weight could be maintained, although malabsorption associated with low serum retinol levels can result in low utilisation of the nutrients and loss of weight (Grunfield & Feingold, 1992; Semba & Tang, 1999; Smith et al., 1992; Whitney et al., 1998).

The mean vitamin A intake of the control group was lower than the DRI's despite the additional 167 µgRE supplied by the multivitamin tablet. These inadequate intakes of vitamin A among the people in the Northwest province are consistent with those found by Vorster et al. (1997). As indicated in the Multicenter AIDS Cohort Study (Tang et al.,

1993) this amount was not sufficient to meet the requirements of 2 727 to 6 060 μgRE (equivalent to 9 000 to 20 000 IU retinol) that was found to reduce the risk of progression during HIV infection. It is appreciated that the control group did not include any AIDS subjects (individuals with a CD4 count less than 200 cells/mm^3). This could be another reason why this particular group experienced the least decrease in both serum retinol and CD4 count with disease progression (Stephensen et al., 1994). Jolly et al. (1997) found that losses of renal function occur in HIV infected patients. In response to infections RBP hastens the loss of vitamin A (Stephensen et al., 1994).

5.3 Serum retinol

In patients with HIV infection the levels of serum vitamin A are reduced as the disease advances (Ward et al., 1993). It is assumed that this effect occurs because more of the vitamin is used up in the effort to maintain the epithelial integrity and to optimize the function of the immune system (Kennedy et al., 2001). In this study the mean level of serum retinol decrease was more or less the same for both study groups. The decreases in the SMMS group ranged from 0.2 – 0.9 μmol/L compared to the decrease of 0.2 – 0.5 μmol/L in the control group. The control group had started with a relatively low mean serum retinol level at 1.8 μmol/L (95% CI: 1.4 -2), which remained constant. After the six months period it remained at 1.7 μmol/L (95% CI: 1.3 –2.1). Several factors could have contributed to the insignificant change seen in the SMMS group. The limitations of the QFFQ are fully acknowledged. Furthermore, the control group did not have subjects with CD4 counts below 200 cells/μL, while the SMMS group had two subjects that fell in this category. However, there were no statistically significant differences in CD4 counts at baseline between experimental and control groups, neither was there a statistically significant difference on changes observed between the two study groups. The former group experienced the least decrease in both serum retinol and CD4 count at a mean of 0.04 μmol/L ($p = 0.05$) and –26 cells/mm^3 ($p>0.05$) respectively, with disease progression. It was also observed in this study that subjects with lower CD4 counts had lower levels of serum retinol (Table 4.6), consequently these two observations could have

contributed to the faster decrease of serum retinol in the SMMP group over the six months period.

Baseline assessment showed that levels of serum retinol were higher than 2μmol/L in subjects with CD4 count ≥ 500 cells/mm^3 (p=0.09). The decrease of serum retinol cells in the SMMP subjects with cell count over 500 was higher than that experienced by those with counts < 500 cells/mm^3. This finding is similar to that of Semba et al. (1996), where subjects with normal serum retinol had CD4 counts over 200 cells/mm^3. In their study the mean CD4 count was 127±37 cells/mm^3 in serum retinol deficient (<1.05μmol/L) adults compared with 237±26 cells/mm^3 in those with normal serum retinol levels. In a study where the population had adequate serum retinol levels supplementation with vitamin A did not affect the rate of progression of the disease. It was only in those with levels below 1.05 μmol/L where the rate of progression from HIV to AIDS decreased with supplementation (Semba et al., 1995[b]). Similarly in this study the serum retinol level of the subjects were in the normal range with the exception of two subjects in the SMMS group and one control, whose levels dropped to 0.8 μmol/L over the six months period. The same subjects also experienced a decrease in CD4 cell count. The two SMMS subjects had a 20% and 48% decrease, while the control subject had a 43% decrease respectively. The initial CD4 count of these three subjects were 428 and 76 for those on SMMP and 766 for the control. However, some of the subjects, with normal serum retinol values in both the intervention and control groups experienced an increase in CD4 cell count (Tables 4.7 and 4.8). This increase is difficult to explain. It could be caused by other confounding variables that were not assessed in this study.

5.4 Immune defense markers

The two immune deficiency markers that will be discussed are the CD4 cell count and the viral load. The former is at times not considered to be reliable because it fluctuates depending on the time of day and the methods used for assessment (New Mexico AIDS InfoNet, 1997), such that the recommendation for using it clearly suggest that the blood samples should be collected at the same time of the day and the same laboratory should be used for analysis (New Mexico AIDS InfoNet, 1997). To limit this possible source of

error and unreliability, both the baseline and end samples in this study were collected around the same time of the day and the baseline samples were stored under controlled conditions and analysed together with the endline samples.

5.4.1 CD4 cell count

The mean CD4 count for both study groups tended to decrease after six months. A significant decrease was seen in the SMMS group ($p<0.01$), and the change in the control group was not significant ($p=0.21$). The intervention group experienced a gradual decline, from a baseline mean of 448 CD4 cells/mm^3. After three months the mean count was 377 cells/mm^3 and at the end of six months it was 333 cells/mm^3. The trend for the control group increased from 424 to 428 cells/mm^3 at midline and decreased to 390 cells/mm^3. Other studies have shown that vitamin A intakes in excess of the RDA (two to four times) can slow the progress of HIV infection to AIDS by enhancing antibody production, even though intakes exceeding the fourfold would diminish the effect (Tang et al., 1993). In this study comparison of dietary vitamin A and serum retinol with the CD4 counts revealed that in the SMMS group the two subjects who had an increase in CD4 count also had increased serum vitamin A. They started with a baseline CD4 count of 263 and 342, which increased by 6.5% and 27% respectively. The end dietary vitamin A intakes (754 and 576μgRE) of these two subjects (both women) from the normal diet were below the DRIs of 900μgRE for women (FNB, 2001). However, two other subjects with an increase in serum vitamin A experienced a decrease in CD4 count, they had baseline counts of 221 and 113, and experienced a –6 and –63% decrease respectively. The former subject did not have a record for the vitamin A intake after six months from the normal diet, but was put on tuberculosis treatment during the study. Her viral load increased by 94%. The latter subject reported intakes of 337 μgRE from the normal diet, and also experienced a 22% increase in viral load. Kennedy et al. (2000) in their review suggest that during HIV infection vitamin A is able to suppress the viral protein, which is required for the reproduction of the virus, thus slowing the progression of the HIV infection. Tang et al. (1997) observed that a decrease of approximately 40 % risk of progression from HIV to AIDS was mediated by moderate daily intakes of dietary

vitamin A (9 000 to 20 000 IU equivalent to 2 727 to 6 060 μgRE), while higher intakes did not have any impact.

In the control group, among the six subjects with increased CD4 counts, three had a rise in serum retinol, two had almost the same levels at both baseline and end, while one had a decrease in this nutrient. These six subjects had dietary intakes below the DRIs with the exception of one, with a mean daily intake of 2 929 μgRE. Except for this subject the mean daily intakes for all subjects (including supplements) were below the 2 727 μgRE threshold which has proved by Tang et al. (1993) to lower the risk to progress from HIV to AIDS. In our study no relationship was observed between the viral load, serum vitamin A and intakes of the nutrient.

Because the CD4 count is at times not a very reliable marker for assessing the progression of HIV infection, in other studies the CD4 as percentage of T-lymphocyte cells is used (New Mexico AIDS InfoNet, 1997). In this study five subjects on SMMS had an increase in the CD4 as a percentage of lymphocytes ranging from 2 to 48%, and five controls also experienced an increase ranging from 12 to 154%. The interesting observation is that only two of the control and one of the SMMS subjects with an increase in CD4 as a percentage of total lymphocytes also had an increase in CD4 cell count.

5.4.2 The viral load

It is expected that in patients with HIV infection as the viral load increases the CD4 count would decrease. Findings from this study support the observation. The SMMS group experienced the highest CD4 count decrease and an increase in viral load. However, three control subjects showed an increase in CD4 cell count, an increase or stable serum vitamin A and a decrease in viral load.

5.4.3 Anthropometric measurements

The SMMS group had a decrease in the initial mean body weight as expected in patients with HIV infection (Niyongabo et al., 1999). However, the group suffered a decrease in fat mass and slightly gained lean body mass. This increase could have been caused by

the dietary supplement that contained a substantial amount of protein at 20g per 100g of the SMMS. The mean daily protein intake of the group from the supplement amounted to 60g. The control group experienced a relatively higher decrease in body weight (loss of 2kg) than the SMMS group, even though they had not progressed into the AIDS stage.

5.5 Quality of life

People that are diagnosed and aware they are infected with HIV usually become very depressed, in addition to this the manifestation of infections associated with the disease also contribute more burden to the stressed body with progression of the condition (Augustus, 1997). Therapy for HIV/AIDS subjects should therefore include social support, as was the case in this study. An improvement in quality of life among both groups was observed. The improvement occurred in the subjects who received the SMMS supplement as well as the control groups with or without the social support therapy. The improvement among the control group (those receiving social support and none) reached statistical significance ($p < 0.01$).

It was indicated earlier in this report that frequent infections were reported by the SMMS group over the six months relative to the control group. This is possibly one of the factors which influenced the relatively higher scores in improvement of quality of life in the latter group. The social support therapy in this study included nutrition education on foods to be eaten and vegetable production. In addition all the study subjects received nutrition education from the trained dietitians. Another randomised controlled intervention study found that within four weeks nutrition counseling with or without supplementation also played an important role in the management of malnourished HIV patients (Rabeneck et al., 1998). The SMMS group started off with a higher score on quality of life, consequently there was less room for improvement compared with the control group.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The purpose of this study was to assess if supplementing the diet of HIV/AIDS patients with SMMP would improve vitamin A status and delay progression into the AIDS stage. The conclusions drawn here will relate to this objective.

6.1.1 The subjects who experienced an increase in CD4 cell count were in both the intervention and control groups, these included those with baseline counts less than 500 cells/mm^3, and those with normal serum retinol levels. Only three subjects (one control and two from the intervention group) had serum retinol levels below normal. This effect could have been caused by other confounding variables that were not assessed by this study. The multivitamin that was taken by both groups could have masked the effect of vitamin A from SMMS.

6.1.2 The SMMS group included AIDS subjects. Their catabolic state, as well as the high occurrence rate of infection required a relatively higher utilization of vitamin A. RBP a reverse acute phase protein also significantly ($p=0.02$) decreased in this group, which is an indication that the level of infections in the group significantly increased. This may be one of the reasons why the level of serum retinol decrease was higher in this group.

6.1.3 Comparison of the level of CD4 T-cell count decrease and serum vitamin A levels among the study groups showed that the control group experienced the least CD4 count decrease and the highest serum retinol relative to the SMMS group. This evidence does not support the theory that vitamin A delays progression of HIV infection, because the vitamin intake of the latter group were higher.

6.1.4 The vitamin A dietary intakes for both groups were lower than the amounts (9 000 to 20 000 μgRE) that have proved by Tang et al. (1993) to slow the progress of HIV infection into the AIDS stage.

6.1.5 A relationship was not established between serum retinol levels and occurrence of infections in this study. All 28 subjects had serum retinol levels >1.05 μmol/L.

In this study the occurrence of infections was notably low and this could be attributed to the adequate food intake reported by the study subjects. In their review Semba & Tang (1999) indicate that decreased food intake can compromise nutritional status and has been associated with adverse clinical outcomes during HIV infection.

6.1.6 The subjects on SMMS maintained their mean lean body mass (not statistically significant).

6.1.7 The study subjects on supplements without social support treatment also experienced an improvement in quality of life. The improvement in the control group was statistically significant ($p<0.01$) and the intervention group $p=0.08$.

6.2 Recommendations

The recommendations made here are related to both the use of SMMS and vitamin A as supplements for persons with the HIV infection.

6.2.1 Considerations should be made to use vitamin A supplemenattion as a public health intervention for HIV/AIDS subjects with low CD4 cell count (<200cells/mm^3) because this group showed low serum retinol levels. Other studies (Kennedy et al., 2000; Hussey et al., 1996; Coutsoudis et al.,1997; Fawzi et al., 1998) also showed an improvement in vitamin A status among subjects with CD4 cell counts <400 cells/mm^3.

6.2.2 An intervention study using a 2 727 to 6 060 µgRE vitamin A supplement should be undertaken, to assess the effect on HIV progression in the developing countries.

6.2.3 The SMMS should be used as another source of vitamin A for HIV patients, even though high amounts have to be eaten to provide the 2 727 µgRE. Considerations to fortify the supplement with vitamin A could be made. This supplement would also provide other nutrients that are essential for the HIV/AIDS patient.

6.2.4 Intervention studies should be done that will take a longer period (such as 12 months) and include a larger study population to assess the effect of SMMS supplementation on HIV disease progression into the AIDS stage. De Pee et al. (1997) studied the relative effects of daily ingestion of vitamin A, and found that for a reliable serum retinol test, the minimal sample size is 19, and 35 for RBP. Contrary to this in a study by Stolzfus et al. (1993) the calculated minimal sample size for detecting a significant effect at three months was 148 for serum retinol. Furthermore, due to failure of HIV subjects to attend their follow-up visits regularly (61.4% in the study of Chikobvu et al., 2000), it is recommended that the size of the sample needed for detecting significant effects, be doubled in future studies. Recruitment of so many subjects is difficult, because of the stigma attached to the disease.

CHAPTER 7

REFERENCES

ABRAMS, B., DUNCAN, D. & HERTZ, PICCIOTTO, I. 1993. A prospective study of dietary intake and acquired immune deficiency syndrome in HIV-seroppositive homosexual men. Journal of acquired immune deficiency syndrome, 6: 949 –958.

AUGUSTUS, L.J. 1997. Nutritional care for patients with HIV. American journal of nursing, 97 (10):62-65. [Available on internet:] htpp://www.nursingcenter.com.

BAUM, M.K., SHOR-POSNER, G., BONVEHI, P., CASSETTI, I., LU, Y., MANTERO-ANETIENZA, E., BEACH, R.S. & SAUBERLICH, H.E. 1993. Influence of infection on vitamin status and requirements. Annals of the New York Academy of Sciences, 669:165 - 174.

CALITZ, J.M. 2000. Provincial population projections, 1996-2021: High HIV/AIDS impact.

CAMP, W.L., ALLEN, S., ALVAREZ, J.D., JOLLY, P.E., WEISS, H.L., PHILLIPS, J.F., KARITA, E., SERUFILIRA, A & VERMUND, S.H. 1998. Serum retinal and HIV1 RNA viral load in rapid and slow progressors. Journal of acquired immune deficiency syndrome human retrovirology, 18(4): 401-406.

CASTETBON, K. KADIO, A., BONDURAND, A., BOKA YAO, A., BAROUAN, C., COULIBALY, Y., ANGLARET, X., MSELLATI, P., MALVY, D & DABIS, F. 1997. Nutritional status and dietary intakes in human immunodeficiency virus (HIV)-infected outpatients in Abidjan, Côte D'Ivoire, 1995. European journal of clinical nutrition, 51:81-86.

CENTERS FOR DISEASE CONTROL. 1997. Revision of CDC surveillance case definition for acquired immunodeficiency syndrome. MMWR, 36 (suppl 1):1S-15S.

CERVINSKAS, J. & LOTFI, M. 1996. Vitamin A deficiency: Key resources in its prevention and elimination. The micronutrient initiative information paper No.1. [Web:] http://www.micronutrient.org/publications/vadsol.shtml [Date of access: 17 June 2000].

CHELIBOWSKI, R.T. 1985. Significance of altered nutritional status in acquired immunodeficiency syndrome (AIDS). Nutrition and cancer, 7:85.

CHIKOBVU, P., STEINBERG, W.J., JOUBERT, G., VILJOEN, J.I., COETZEE, M., KRIEL, J. & VAN DER RYST, E. 2000. Lessons learned in establishing a randomized controlled trial to investigate the effect of vitamin A on vertical transmission of HIV-1. The Southern African journal of epidemiology and infection, 15(1):19-22.

COUTSOUDIS, A., MOODLEY, D., PILLAY, K., Effect of vitamin A supplementation on viral load in HIV-1 infected pregnant women. 1997. Journal of immune deficiency syndrome, 15:86-87.

DE HOOP, M.E. & MATJI, J. .2000. The South African Department of Health: Long term strategies to eliminate vitamin A deficiencies. (*In* Long–term food based approach towards eliminating vitamin A deficiency in Africa: Papers read at the workshop organised by the South Africa Medical Research Council, the United Nations University (UNU) and the International Union of Nutritional Sciences (IUNS) in Cape Town on 22-23 November 2000. Cape Town. p.10.).

DE LUCA, L.M., DARWICHE, N., CELLI, G., KOSA, K., JONES, C., ROSS, S. & CHEN, L. 1994. Vitamin A in the epithelial differentiation and skin carcinogenesis. Nutrition reviews, 52:2.

DE PEE, S., YUNIAR, Y., WEST, C.E. & MUHILAL. 1997. Evaluation of biochemical indicators of vitamin A status in breast-feeding and non-breast-feeding Indonesian women. American journal of clinical nutrition, 66:160-170.

DE WAGT, A. 2000. Vitamin A deficiency control programmes in Eastern and Southern Africa. (*In* Long–term food based approach towards eliminating vitamin A deficiency in Africa: Papers read at the workshop organised by the South Africa Medical Research Council, the United Nations University (UNU) and the International Union of Nutritional Sciences (IUNS) in Cape Town on 22-23 November 2000. Cape Town. p.6.)

DEPARTMENT OF HEALTH, NORTH WEST PROVINCE. 1998. Results of the HIV sero-prevalence survey of women attending public antenatal clinics in the North West Province. Mmabatho:DOH

DOWLING, S., MULCAHY, F. & GIBNEY, M.J. 1990. Nutrition in the management of HIV antibody positive patients: A longitudinal study of dietetic out-patient advice. European journal of clinical nutrition, 44:823-829.

FABRIS, N., MOCCHEGIANI, E., IRATO, L., LAZZARIN, A. & MORONI, M. 1988. AIDS, zinc deficiency and thymic hormone failure. JAMA, 259:839.

FALUTZ, J., TSOUKA, C. & GOLD, P. 1988. Zinc as a cofactor in human immunodeficiency virus-induced immunosuppression. JAMA, 259:2850.

FAWZI, W.W., MSAMANGA, G.I., SPIEGELMAN, D., URASSA, E.N.J., McGRATH, N., MWAKAGILE, D., ANTELMAN, G., MBISE, R., HERRERA, G., KAPIGA, S., WILLET, W.& HUNTER, D.J. 1998. Randomised trial of effects of vitamin A supplement on pregnancy outcomes and T cell counts in HIV-1 infected women in Tanzania. Lancet, 351:1477-1482.

FOOD AND NUTRITION BOARD, INSTITUTE OF MEDICINE. 1989. Recommended dietary allowances, 10th ed. Washington DC: National Academy Press.

FOOD AND NUTRITION BOARD, INSTITUTE OF MEDICINE. 2001. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington, D.C.: National Academy Press.

FRIIS, H. & MICHAELSEN, K.F. 1998. Micronutrients and HIV infection: A review. European journal of nutrition, 52: 157-163.

FRIIS, H., GOMO, E., KAESTEL, P., NDLOVU, P., NYAZEMA, N., KRARUP, H. & MICHAELSEN, K.F. 2001. HIV and other predictors of serum β-carotene and retinol in pregnancy: a cross-sectional study in Zimbabwe. American clinical journal of nutrition, 73:1058-1065.

GRUNFELD, C. & FEINGOLD, K.R. 1992. Metabolic disturbances and wasting in the acquired immunodeficiency syndrome. The New England journal of medicine, 327(5):329-337.

HALL, C.S. 1989. Food programs: Assessing and developing community-based nutritional support for people with HIV-related illness. Washington, DC: National AIDS Network.

GOLDBERG, G.R., BLACK, A.E., JEBB, S.A., COLE, T.J. MURGATROYD, P.R., COWARD, W.A. & PRENTICE, A.M 1991. Critical evaluation of energy intake data using fundamental principles of energy physiology: I Derivation of cut-off limits to identify under-recording. European journal of clinical nutrition, 45: 561-581.

HANSON, P. 1996. Deficiencies of vitamin A in a normal diet in Lesotho: Study on the effect of traditional processing methods on vitamin A loss. Grimsby : University of North Lincolnshire. (Dissertation – BSc Hons.).

HEALTH SYSTEMS AGENCY. 1993. Perspectives. Jan/Feb: 17 (1).

HUSSEY, G., HUGHES, J. & POTGIETER, S. 1996. Vitamin A status and supplementation and its effects on immunity in children with AIDS. (*In* Abstracts of the XVII International Vitamin Consultative Group Meeting, Guatemala City. Washington, DC: International Life Sciences Institute, p.6.)

JOLLY, P.E., MOON, T.D., MITRA, A.K., DEL ROSARIO, G.R., BLOUNT, S. & CLEMONS, T.E. 1997. Vitamin A depletion in hospital and clinic patients with acquired immunodeficiency syndrome – a preliminary report. Nutrition research, 17 (9):1427-1441.

KENNEDY, C.M., KUHN, L. & STEIN, Z. 2000. Vitamin A and HIV infection: Disease progression, mortality and transmission. Nutrition reviews, 58(10): 291-302.

LABADARIOS, D. 2001. National Food Consumption Survey, 1999. South African journal of clinical nutrition, 14 (2): 62 – 75.

LACEY, C., MURPHY, E., SANDERSON, MARION, MONTEIRO, E.F., VAIL, A. & SCHORAH, C. J. 1996. Antioxidant-microtrients and HIV infection. International journal of STD & AIDS, 7:485-489.

LANGENHOVEN, M.J., KRUGER, M., GOUWS, E. & FABER, M. 1991. MRC food composition tables. 3rd ed. Tygerberg: Medical Research Council. 245p.
LUBBE, A.M. & MAREE, C.M. 1973. Dietary survey in the Mount Ayliff district. A preliminary report. South African medical journal, 45:304-307.

MACINTYRE, U.E. 1998. Dietary intakes of Africans in transition in the North West Province. Potchefstroom: Potchefstroom University for Christian Higher Education. (Thesis-Ph.D.) 450p.

MACINTYRE, U.E., KRUGER, H.S., VENTER, C.S. & VORSTER, H.H. 2001. Dietary intakes of an African population in different stages of transition in the NorthWest Province, South Africa: The THUSA study. Journal of human nutrition and dietetics (in press)

MAHAN, L.K. & ARLIN, M. 2000. Krause's food, nutrition & diet therapy. 10th ed. Philadelphia:Saunders.

McCORNKIDALE, C. 1990. Nutritional status of HIV-infected patients during the early disease stages. Journal of the American Dietetic Association, 90:1236-1241.

MULLIGAN, K., TAI, V.W. & SCHAMBELAN, M. 1997. Energy expenditure in human immunodeficiency virus. New England journal of medicine, 336:70.

NAKASHIMA, H., OKUBO, K., HONDA, Y., TAMURA, T., MATSUDA, S. & YAMAMOTO, N. 1989. Inhibitory effect of glycosides like saponins from soybean on the infectivity of HIV in vitro. AIDS, 3 (10): 655-658.

NIYONGABO, T., HANZEL, D., NDAYISHIMYIE, J.M., MELCHIOR, J.C., NDAYIRAGIJE, A., NDIHOKUBWAYO, J.B., NIKOYAGIZE, E., RWAZA, T., AUBRY, P. & LAROUZE, B. 1999. Nutritional status of adult inpatients in Bujumbura, Burundi (impact of HIV infection). European journal of nutrition, 53:579-582.

NEW MEXICO AIDS INFONET. 1997. T-cell tests. [Web:] http://www.aidsinfonet.org [Fact sheet number 412].

PEAKMAN, M. & VERGANI, D. 1997. Basic and clinical immunology. New York: Churchill Livingstone.

POLIT, D.F. & HUNGLER, B.P. 1987. Nursing research:principles and methods. 3rd ed. Philadelphia:Lippincott.

RABENECK, K. et al. 1998. A randomized controlled trial evaluation of nutrition councelling with or without oral supplementation in malnourished HIV infected patients. Journal of American Dietetic Association, 98:433

RICH, K.C., FOWLER, M.G., MOFENSON, L.M. 2000. Maternal and infant factors predicting disease progression in human immunodeficiency virus-type 1-infected infants. Women and Children Transmission Study group. Pediatrics,105: E8.

SAVACG (South African Vitamin A Consultative Group). 1995. Children aged 6 to 71 months in South Africa, 1994: Their anthropometric, vitamin A, iron and immunization coverage status. Isando: SAVACG.

SEMBA, R.D., GRAHAM, N.M.H., CAIAFFA, W.T., MARGOLICK, J.B., CLEMENT, L. & VLAHOV, D. 1993. Increased mortality associated with vitamin A deficiency during human immunodeficiency virus type 1 infection. Archives of internal medicine, 153:2149-2154.

SEMBA, R.D. 1994. Vitamin A, immunity, and infection. Clinical infectious diseases, 19:489-499.

SEMBA, R.D., MIOTTI, P.G., CHIPHANGWI, J.D., LIOMBA, G., YANG, L.P., SAAH, A., DALLABETTA, G.A. & HOOVER, D.R. 1995[a]. Infant mortality and martenal vitamin A deficiency during human immunodeficiency virus infections. Clinical infectious diseases, 21: 966 – 972.

SEMBA, R.D., CAIAFFA, W.T., GRAHAM, N.M., COHON, S. & VLAHOV, D. 1995[b]. Vitamin A deficiency and wasting as predictors of mortality in human immunodeficiency virus-infected drug users. Journal of infectious diseases, 17: 1196 – 1202.

SEMBA, R.D., PARK, S., ROYAL, W. & GRIFFIN, D.E. 1996. Vitamin A deficiency and T-cell sub-populations in HIV-infected adults. Nutrition research, 16(6):915-923.

SEMBA, R.D., MIOTTI, P., CHIPANGWI, J.D., 1997. Maternal vitamin A deficiency and child growth failure during human immunodeficiency virus infection. Journal of acquired immune deficiency syndrome human retrovirology, 14: 219 – 22.

SEMBA, R.D. 1998. The role of vitamin A and related retinoids in immune function. Nutrition reviews, 56(1):S38-S48.

SEMBA, R.D. & TANG, A.M. 1999. Micronutrients and the pathogenesis of human immunodeficiency virus infection. British journal of nutrition, 81:181-189.

SMITH, P.D., QUINN, T.C., STROBER, W., JANOFF, E.N. & MASUR, H. 1992. Gastrointestinal infections in AIDS. Annals of internal medicine, 116:63-77.

STEPHENSEN, C.B., ALVAREZ, J.O., KOHATSU, J., HARDMEIER, R., KENNEDY, J.I. Jr & GAMMON, R.B. Jr. 1994. Vitamin A is secreted in the urine during acute infection. American journal of clinical nutrition, 60:388-392.

STOLTZFUS, R.J., HABICHT, J.P., RASMUSSEN, K.M. & HAKIMI, M. 1993. Evaluation of indicators for use in vitamin A intervention trials targeted at women. International journal of epidemiology, 22:1111-1118.

STOLTZFUS, R.J. & KLEMM, R. 1997. Sustainable control of vitamin A deficiency: Defining progress through assessment, surveillance, evaluation. Report of the XVIII International Vitamin A Consultative Group meeting. Cairo, Egypt.

TANG, A.M., GRAHAM, N.M.H., KIRBY, A.J., MCCALL, D.L., WILLET, W.C. & SAAH, A.J. 1993. Dietary micronutrient intake and risk of progression to acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus type 1 (HIV - 1)- infected homosexual men. American journal of epidemiology, 138 (11): 937 - 951.

TANG, A.M., GRAHAM, M.H. & SAAH, A.J. 1996. Effects of micronutrient intake on survival in human immunodeficiency virus type 1 (HIV-1)-infected homosexual men. American journal of epidemiology, 143: 1244 –1256.

TANG, A.M., GRAHAM, M.H., CHANDRA, R.K. & SAAH, A.J. 1997. Low serum vitamin B12 concentrations are associated with faster human immunodeficiency virus type 1 (HIV-1) disease progression. Journal of nutrition,127: 345-351.

THURNHAM, D.I., 1989. Vitamin A deficiency and its role in infection. Trans Royal Society Tropical Medicine and Hygiene, 83: 721 –723.

ULLRICH, R., SCHNEIDER, T., HEISE, W., PAGE, M., HOFFKEN, G. & REEEKEN, E.O. 1994. Serum carotene deficiency in HIV-infected patients. AIDS, 8: 661 – 665.

UNDERWOOD, B.A. & OLSON, J.A. 1993. A brief guide to current methods as assessing vitamin A status: A report of the International Vitamin A Consultative Group (IVACG). Washington D.C.

VAN STADEN, A.M., BARNARD, H.C., NEL, M., ATTWOOD, E.M., OOSTHUIZEN, G.M., DANHAUSER, A., VAN DER RYST, E. & LE ROUX, G.D. 1998. Nutritional status of HIV-1 seropositive patients in the Free State province of South Africa – laboratory parameters. Central African journal of medicine, 44(10): 246 – 252.

VENTER, C.S., MACINTYRE, U.E. & VORSTER, H.H. 2000. The development and testing of a food portion photograph book for use in an African population. Journal of human nutrition and dietetics, 13: 205-218.

VITAMIN INFORMATION CENTRE. 1998. Micronutrients: Their role in the management of the AIDS patient. Medical update, 31.

VORSTER, H.H., MARGETTS, B., VUNDULE, C., VENTER, C.S., KRUGER, H.S. & VELDMAN, F.J. Unpublished. The metabolic profiles of asymptomatic HIV positive Africans: Direction for dietary intervention?

VORSTER, H.H., OOSTHUIZEN, W., JERLING, J.C., VELDMAN, F.J.& BURGER, H.M. 1997. The nutritional status of South Africans: A review of the literature from 1975-1996, reference tables. Potchefstroom: Health systems trust.

VORSTER, H.H., WISSING, M.P.,., VUNDULE, C., VENTER, C.S., KRUGER, H.S. & VELDMAN, F.J, MARGETTS, B & U, MACINTYRE. 2000. The impact of urbanization on physical, physiological and mental health on Africans in the North West Province of South Africa: the THUSA study. South African journal of science, 96:505-514.

VORSTER, H. 2001. Ethical considerations surrounding HIV/AIDS: How do the health professionals of South Africa penetrate the brick wall they are facing?. Woord & daad – Herfs, Autumn:11 – 19.

WARD, B.J., HUMPHREY, J.H., CLEMENT, L. & CHAISSON, R.E.1993. Vitamin A satus in HIV infection. Nutrition research, 13:157-166.

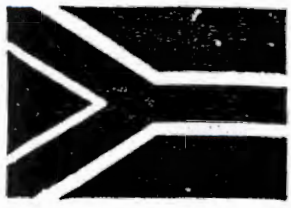
WHITNEY, E.N., CATALDO, C.B. & ROLFES, S.R. 1998. Understanding normal and clinical nutrition. 5th ed. Minneapolis:West.

WISSING, M.P., THEKISO, S., VAN QUICKELBERGE, L., CHOABI, P., MOROENG, C. & NIENABER, A.W. 1999. The psychometry properties of scales measuring psychological well-being in an African group. International Africa Psychology Congress, 18-23 July 1999, Durban, South Africa.

ZHANG, R., YAPING, L. & WANG, W. 1997. Enhancement of immune function in mice fed high doses of soy diadzein. Nutrition and cancer, 29 (1): 24-28.

Appendix A

Republic of South Africa



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20 July 1999

Prof Hester H. Vorster
Nutrition Department
Potchefstroom Univeristy
P.Bag X 6001
Potchefstroom 2520

Fax: 018-2992799

Dear Prof Vorster,

RE: PERMISSION TO CONDUCT RESEARCH IN THE NORTH WEST PROVINCE

The Departmental Research Committee recently reviewed your research proposal entitled ***A randomised double blind controlled study of oral anti-oxidants and phytochemical supplementation of African HIV/AIDS patients,*** and wishes to inform you that permission has been granted for you to conduct your study, subject to the following conditions:

i. The Ethics/Research Review Committee of your academic institution has approved your proposal,

ii. The Department will not be responsible for any costs associated with the research project,

iii. That on completion of the research project, a copy of your research report (or dissertation or thesis) will be submitted to the Department.

Attached are comments from the Departmental Research Committee that you should address before conducting your research project.

Any enquiries and correspondence regarding the research should be addressed to Mr Caesar Vundule or Mrs Rebone Gcabo (tel. (018) 3875213/6).

Yours sincerely,

M.C. NTOANE
Deputy Director General

INFORMED CONSENT : HIV RESEARCH PROJECT FORM (ETHICS NO: 99M04)

I, the undersigned, _____ have been informed regarding the project and I declare that I understand it. I have been given the chance to discuss aspects of the project with the project leader and declare that I am willing to take part in the project. Hereby I give my consent to act as a subject in the project, and am willing to disclose my identity in a group.

I thereby indemnify the University and any employee or student of the University against any liability which may arise during the project. I undertake further that no claim will be made against the University for damage, which I may suffer due to the project, whether it is due to negligence of the University, its employees or students or other project subjects.

_____

(Signature of project subject)

Signed at _____ on _____

Witnesses:

1. _____

2. _____

Signed at _____ on _____

The HIV 2000 project

Date_____

Place_____

Subject name: ..... Subject no: .....

Signature

STATION 1	Demographic questionnaire	.....
STATION 2	Blood Samples Clinical signs: skin ..... mouth ..... diarrhoea ..... TB ..... Other ..... Blood pressure ..... Oral temperature ..... Medication .... Blood samples:	 mm Hg ° C
STATION 3	Anthropometry and activity questionnaire Weight..... kg height ..... m waist .....cm hip.....cm triceps.....mm sub-scapula.....mm	 .....
STATION 4	Quality of life	.....
STATION 5	Family Support Programme	.....
STATION 6	Dietary questionnaire	.....
STATION 7	Dietary education, supplement, next appointment, calender, station card, busfare (R10), snacks Supplements received: Multivitamin Sportron A Sportron B SPP	 ....

FOOD INTAKE DIARY FOR THE NUTRITION 2000 STUDY

NAME OF CLIENT ..... DATE OF ISSUE..... RECORD NUMBER .....

DATE	TIME	FOOD TAKEN	AMOUNT OF MAIZE- SOYA SUPPLEMENT EATEN	DRUGS TAKEN	GENERAL FEELING
Example 01.03.2000	**Morning**	Tea with sugar and milk, Bread with peanut butter,		Vitamin Bco	Bloated
	Mid-morning	Orange	150g or Half a packet	1 Multivitamin	Mild abdominal pains
	Lunch	Pap, Beef stew, spinach, water			
	Mid-afternoon	Water	150g or half a packet	I teaspoon multivitamin syrup	
	Supper	Pap, and beans			
Day 1	**Morning**				
	Mid-morning				
	Lunch				
	Afternoon				
	Supper				

Appendix D

AUGUST

Sun	Mon	Tue	W
		1 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	2 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes
6 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	7 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	8 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	9 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes
13 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	14 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	15 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	16 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes
20 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	21 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	22 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	23 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes
27 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	28 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	29 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes 	30 Mouth sores Diarrhoea Vomiting Cough Fever Skin Rashes

2000

Thu	Fri	Sat
3 Next appointment clinic	4 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes	5 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes
10 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes	11 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes	12 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes
17 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes	18 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes	19 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes
24 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes	25 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes	26 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes
31 Mouth sores, Diarrhoea, Vomiting, Cough, Fever, Skin Rashes		

Subject number_____ **Interviewer**_____

QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

INTRODUCTION:

Greeting

Thank you for giving up your time to participate in this study. I hope you are enjoying it so far. Here we want to find out what people living in this area eat and drink. This information is important to know as it will tell us if people are eating enough and if they are healthy.

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

- if you eat the food
- how the food is prepared
- how much of the food you eat at a time
- 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 you eat it.

To help you to describe the amount of a food you eat, I will show you pictures of different amounts of the food. Please say which picture is the closest to the amount you eat, or if it is smaller, between sizes or bigger than the pictures.

THERE ARE NO RIGHT OR WRONG ANSWERS.

EVERYTHING YOU TELL ME IS CONFIDENTIAL. ONLY YOUR SUBJECT NUMBER APPEARS ON THE FORM.

IS THERE ANYTHING YOU WANT TO ASK NOW?

ARE YOU WILLING TO GO ON WITH THE QUESTIONS?

INSTRUCTION

Circle the subject's answer. Fill in the amount and times eaten in the appropriate columns.

I shall now ask you about the type and the amount of food you have been eating in the last few months. Plea tell if you eat the food, how much you eat and how often you eat it. We shall start with maize meal porridge.

Do you eat maize meal porridge? **YES** 1 **NO** 2

If YES, what type do you have at home now?

Brand name_____

Don't know_____2

Grind self_____3

If brand name given, do you usually use this brand **YES** 1 **NO** 2 **DON'T KNOW** 3

Where do you get your maize-meal from? (May answer more than one)

Shop ☐ 1

Employer ☐ 2

Harvest and grind self ☐ 3

Other - specify _____ ☐ 4

Don't know ☐ 5

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/E
			Per day	Per week	Per month	Seldom/ Never		
Maize-meal porridge	Stiff (pap)						e4225 4250	
Maize-meal porridge	Soft (slappap)						e4225 4250	
Maize-meal porridge	Crumbly (phutu)						e4225 4250	
Ting								
Mabella Coarse Fine Rice	Stiff						4082	
Mabella	Soft						4082	
Oats							4032	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DA
			Per day	Per week	Per month	Seldom/ Never		
Breakfast cereals	Brand names of cereals at home now: (5) _ Don't know							

Do you pour milk on your porridge or cereal? YES 1 NO 2

If YES, what type of milk (whole fresh, sour, 1%, fat free, milk blend.)_____

INSTRUCTION: Show subject examples.

If YES, how much milk?								

Do you pour sugar on your cereal/porridge/mabella YES 1 NO 2

If YES, how much sugar?							9012	
Samp	Bought Self ground						4077 4073	
Samp and beans							A014	

Are the amounts of samp and beans the same as in the picture? YES NO

If no, do you use more beans than in the picture or less? MORE LESS

Samp and peanuts							A013	

Are the amounts of samp and peanuts the same as in the picture? YES NO

If no, do you use more peanuts than in the picture or less? MORE LESS

Rice	White Brown Maize rice						4040 4134 4043	
Pastas	Macaroni Spaghetti Other:						4062	

You are being very helpful. Can I now ask you about meat?

CHICKEN, MEAT, FISH Hoeveel keer in 'n week - eet jy vleis?

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/D
			Per day	Per week	Per month	Seldom/ Never		
Chicken	Boiled						1521	
	Fried: in batter/crumbs Not coated						1634 1520	
	Roasted/grilled						1520	

Do you eat chicken skin **ALWAYS** 1 **SOMETIMES** 2 **NEVER** 3

Chicken bones stew							A003	
Chicken feet							A004 1609	
Chicken offal							1610	
Red meat:	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed						A001	
	Mince with tomato and onion						1585	
Beef Offal	Intestines: boiled, nothing added						1616	
	Stewed with vegetables							
	Liver						1515	
	Kidney						1518	
	Other specify:							
What vegetables are usually put into meat stews?								
Wors / sausage	Fried						1526	
Bacon							1501	
Cold meats	Polony						1514	
	Ham						1564	
	Viennas						1531	
	Other - specify							
Canned meat	Bully beef						1535	
	Other specify:							
Meat pie	Bought						1548	
Hamburger	Bought						A015	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DA
			Per day	Per week	Per month	Seldom/ Never		
Dried beans/peas/ lentils (10)	Soup Salad						3033 3508	
Soya products eg. Toppers	Brands at home now (5) Don't know_____ Show examples						3527	
Pilchards in tomato/chilli/ brine	Whole						2557	
	Mashed with fried onion						A005	
Fried fish	With batter/crumbs						2509	
	Without batter/crumbs						2523	
Other canned fish	Tuna						2547	
	Pickled fish Other:						2562	
Fish cakes	Fried						2531	
Eggs	Boiled/poached Scrambled Fried						1001 1025 1003	

WE NOW COME TO VEGETABLES AND FRUIT

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/D
			Per day	Per week	Per month	Seldom/ Never		
Cabbage	How do you cook cabbage?							
	Boiled, nothing added						8066	
	Boiled with potato and onion and fat						A096	
	Fried, nothing added						A097	
	Boiled, then fried with potato, onion						A006	
	Other: Don't know							

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DA
			Per day	Per week	Per month	Seldom/ Never		
Spinach/morogo/ other green leafy	How do you cook spinach?							
	Boiled, nothing added						8071	
	Boiled fat added						8209	
	Boiled with onion/tomato and fat						A011	
	- onion, tomato & potato							
	- with peanuts							
	Other: Don't know							
Tomato and onion 'gravy'	Home made - with fat - without fat						A012 A016	
	Canned						8221	
Pumpkin	How do you cook pumpkin?							
	Cooked in fat & sugar						A010	
	Boiled, little sugar and fat						A009	
	Other: Don't know							
Carrots	How do you cook carrots?							
	Boiled, sugar & fat						8129	
	With potato/onion						A008	
	Raw, salad						8015	
	Chakalaka							
	Other: Don't know							
Mealies/Sweet corn	How do you eat mealies?							
	On cob						8033	
	Off cobb - creamed sweet corn - whole kernel						8034 8261	
Beetroot salad	Home made Bought						8005	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Potatoes	How do you cook potatoes?							
	Boiled/baked - with skin						8046	
	- without skin						8045	
	Mashed						8187	
	Roasted						8189	
	French fries						8048	
	Salad Other:						8236	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						8057	
	- without skin						8214	
	Mashed							
	Other: Don't know							
Salad vegetables	Raw tomato						8059	
	Lettuce						8031	
	Cucumber						8025	
Other vegetables, specify:								

FRUIT:

Do you like fruit? YES NO

Apples/Pears	Fresh Canned pears						7001 7054	
Bananas							7009	
Oranges/naartjie							7031	
Grapes							7020	
Peaches	Fresh Canned						7036 7038	
Apricots	Fresh Canned						7003 7004	
Mangoes	Fresh						7026	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DA
			Per day	Per week	Per month	Seldom/ Never		
Guavas	Fresh Canned						7021 7023	
If subject eats canned fruit: Do you have custard with canned fruit:			YES 1		NO 2			
Custard	Home made Ultramel						0004	
Wild fuit/berries	Specify type						7070	
Dried fruit	Types:							
Other fruit								

BREAD AND BREAD SPREADS

Bread/Bread rolls	White						4001	
	Brown						4002	
	Whole wheat						4003	
Do you spread anything on the bread?			ALWAYS 1		SOMETIMES 2		NEVER 3	
Margarine	What brand do you have at home now? _____ Don't know_____ **Show examples**							
Peanut butter							6509	
Jam/syrup/honey							9008	
Marmite/Fray Bentos							9501	
Fish/meat paste							1512	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/D
			Per day	Per week	Per month	Seldom/ Never		
Cheese	Type:						0010	
Achaar							A017	
Other spreads:	Specify							
Dumpling							4001	
Vetkoek							4057	
Provita, crackers, etc.								
Mayonnaise/salad dressing	Number of spoons_____ number in family						6573	

DRINKS:

Tea							9514	
Coffee							9513	
Sugar/cup tea or coffee							9012	
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life whole						0006	
	Fresh/long life 2%						0069	
	Fresh/long life fat free						0072	
	Whole milk powder Brand						0009	
	Skimmed milk powder Brand						0008	
	Milk blend Brand						0068	
	Whitener Brand						0039	
	Condensed milk						0002	
	Evaporated milk						0003	
	None							
Milk as such	What type of milk do you drink as such?							

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DA
			Per day	Per week	Per month	Seldom/ Never		
	Fresh/long life whole						0006	
	Sour / Maas						0006	
Milk drinks Brand	Nestle_____ Milo_____ Flavoured milk_____ Other_____						0023	
Yoghurt	Drinking yoghurt Thick yoghurt						0044 0020	
Squash	SweetO SixO Oros/Lecol with sugar - artificial sweetener Kool Aid Other						9013 9013 9002 9013 9002	
Fruit juice	Fresh/Liquifruit/Ceres						0535	
	Tropica Show examples						0089	
Fizzy drinks Coke, Fanta	Sweetened Diet						9001 9013	
Mageu/Motogo							9562	
Home brew							9516	
Tlokwe							9516	
Beer							9506	
Spirits							9510	
Wine red							9508	
Wine white							9518	
Other specify								

SNACKS AND SWEETS:

Potato crisps							8049	
Peanuts	Raw Roasted						6001 6007	
Cheese curls: Niknaks etc.							4076	
Raisins							7022	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DA
			Per day	Per week	Per month	Seldom/ Never		
Peanuts and raisins							6007 7022	
Chocolates	Name						9024	
Candies	Sugus, gums, hard sweets						9009	
Sweets	Toffees, fudge, caramels						9014	
Biscuits	Type							
Cakes & tarts	Type							
Scones							4029	
Rusks							4160	
Savouries	Sausage rolls Samoosas Biscuits eg bacon kips Other:						1534 4196 4162	
Jelly							9004	
Baked pudding							4181	
Instant pudding							4066	
Ice cream Sorbet							6507 6516	
Other Specify:								

SAUCES / GRAVIES / CONDIMENTS

Tomato Sauce Worcester sauce							9505	
Chutney							9524	
Pickles							8176	
Packet soups							4069	
Others:								

WILD BIRDS, ANIMALS OR INSECTS (hunted in rural areas or on farms)

Wild fruit								

MISCELLANEOUS: Please mention any other foods used more than once/two weeks which we have not talked about:

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

SALT USE:

What type of salt do you use?_____

The next few questions are to find out if you use salt, where you use it and how much you use?

Do you add salt to food while it is being cooked?

Always	Sometimes	Never	Don't know
1	2	3	4

Do you add salt to your food after it has been cooked?

Always	Sometimes	Never
1	2	3

Do you like salty foods eg. salted peanuts, crisps?

Very much	Like	Not al all
1	2	3

Do you use any of the following:

	Name of product	Amount/da
Vitamins/vitamins & minerals		
Tonics		
Health foods		
Body building preparations		
Dietary fibre supplement		
Other: specify		

THANK YOU FOR YOUR COOPERATION AND PATIENCE

GOOD-BYE!

24-HOUR RECALL

Subject number: _____ Interviewer: _____

Name: _____ _____ __ Date: ____/ _____/ 200___

Tick what the day was yesterd-

Monday	Tuesday	Wednesday	Thursday	Friday

Would you describe the food that you ate yesterday as typical of your habitual food intake?

Yes	1	No	2

If not, why? _____

I want to find out about everything you ate or drank yesterday, including food you pick from the veld. Please tell me everything you ate from the time you woke up to the time you went to sleep. I will also ask you where you ate the food and how much you ate.

Time (approximately)	Place (Home, school, etc)	Description of food and preparation method	Amount	Amount in g (office use only)	Code (office use only)
From waking up to going to work, or starting day's activities					
During the morning at work or at home					

Time (approximately)	Place (Home, school, etc)	Description of food and preparation method	Amount	Amount in g (office use only)	Code (office use only)
Middle of the day (Lunch time)					
During the afternoon					
At night (dinner time)					

Time (approximately)	Place (Home, school, etc)	Description of food and preparation method	Amount	Amount in g (office use only)	Code (office use only)
After dinner, before going to sleep					

• Do you take any vitamins (tablets or syrup)?	Yes	1	No	2
Give the brand name and dose of the vitamin/tonic:				
• Do you receive a mealie meal mix (PVM) at the clinic?	Yes	1	No	2
How often do you eat this?	Daily	Weekly	Monthly	
How much do you eat at a time?				
• Do you receive PVM drink mix at the clinic?	Yes	1	No	2
How often do you eat this?	Daily	Weekly	Monthly	
How much do you eat at a time?				

For office use only		
Card Number	0	9

AFFECTOMETER (AFM)

Instructions

This inventory consists of 20 sentence items. Read each sentence and decide how often the feeling was present over the past few weeks, according to the following graded response scale:

Not at all	Occasionally	Some of the time	Often	All the time
1	2	3	4	5

You are to mark your answer next to each statement as it applies to you. Please answer every statement

1	My life is on the right track	1	2	3	4	5
2.	I wish I could change some part of my life	1	2	3	4	5
3.	My future looks good.	1	2	3	4	5
4.	I feel as though the best years of my life are over	1	2	3	4	5
5.	I like myself	1	2	3	4	5
6.	I feel there must be something wrong with me.	1	2	3	4	5
7.	I can handle any problems that come up.	1	2	3	4	5
8.	I feel like a failure.	1	2	3	4	5
9.	I feel loved and trusted	1	2	3	4	5
10.	I seem to be left alone when I don't want to be.	1	2	3	4	5
11.	I feel close to people around me.	1	2	3	4	5
12.	I have lost interest in other people and don't care about them	1	2	3	4	5
13.	I feel I can do whatever I want to.	1	2	3	4	5
14.	My life seems stuck in a rut.	1	2	3	4	5

15.	I have energy to spare	1	2	3	4	5
16.	I can't be bothered doing anything.	1	2	3	4	5
17.	I smile and laugh a lot.	1	2	3	4	5
18.	Nothing seems very much fun any more.	1	2	3	4	5
19.	I think clearly and creatively.	1	2	3	4	5
20.	My thoughts go around in useless circles.	1	2	3	4	5

CALCULATIONS FOR RATIO OF ENERGY INTAKE (EI) TO BASAL METABOLIC RATE (BMR)

Example of estimation for BMR

Equation for calculation of BMR (Whitney et al.,1998)

For males

Age in years	Equation
3 to 9	(22.7 x body weight) + 495
10 to 17	(11.5 x body weight) + 651
18 to 29	(15.3 x body weight) + 679
30 to 60	(11.6 x body weight) + 879
>60	(13.5 x body weight) + 487

For females

Age in years	
3 to 9	(22.5 x body weight) + 499
10 to 17	(12.2 x body weight) + 746
18 to 29	(14.7 x body weight) + 496
30 to 60	(8.7 x body weight) + 829
>60	(10.5 x body weight) + 596

Therefore for subject number 1 the calculation is

(14.7 x 56.5) + 496 = 1326.6 kJ.

EI/BMR ratio from QFFQ and 24 hour recalls for SMMS subjects

Subject number	24hr recall		QFFQ	
	EI/BMR	Ratio	EI/BMR	Ratio
1	11949/1326.6	9	17300/1326.6	13
18	6098/1360.4	4.5	5472/1360.4	4
20	3218/1511.8	2	112117/1511.8	7.4
22	ND	ND	23654/1707.2	13.9
24	9540/1306	7	16286/1306	12.5
25	4956/1097	4.5	10419/1097	9.5
27	4491/1364.8	3.3	15989/1364.8	11.7
72	1175/1275	0.9	6933/1275	5.4
74	5603/1401	3.4	18756/1401	13.4
76	9668/1748.5	5.5	9668/1748.5	5.5

83	8017/1602.9	5	9719/1602.9	6
85	4035/1340.6	3	11687/1340.6	8.7
86	8732/1472	5.9	13678/1472	9.3
102	4725/1451.5	3.3	8936/1451.5	6.2
103	10643/1457.8	7.3	12801/1457.8	8.8
104	4021/1461.8	2.8	10741/1461.8	7.3

EI = energy intake, BMR = basal metabolic rate, ND= not determined

Note that an EI:BMR below 1.2 is regarded as too low an intake for maintenance of body weight, hence it is considered as under reporting (Goldberg et al., 1991).

EI:BMR ratio from QFFQ and 24 hour recalls for controls

Subject number	**24hr recall**		**QFFQ**	
	EI/BMR	Ratio	EI/BMR	Ratio
12	3852/1486.8	2.6	11151/1486.8	7.5
13	3529/1383.6	2.6	10720/1383.6	7.7
14	2224/1319.7	1.7	13714/1319.7	10.4
17	3984/1136.9	3.5	10967/1136.9	9.6
35	8921/1260.5	7	6203/1260.5	4.9
40	3808/1253.6	3	7644/1253.6	6
51	7976/1210.9	6.6	4633/1210.9	3.8
58	9063/1372.8	6.6	13466/1372.8	9.8
60	5143/1452.8	3.5	9039/1452.8	6.2
64	12079/1702.6	7	21233/1702.6	12.5
95	2454/1307.5	1.9	9915/1307.5	7.6
	3995/1247	3.2	8129/1247	6.5