# SUPPLEMENT CONSUMPTION AND ENERGY INTAKE OF HIV+ CHILDREN RECEIVING AN ENZYME-MODIFIED, ENRICHED MAIZE SUPPLEMENT

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# ABBREVIATIONS

| | |
|---|---|
| 3TC | Lamivudine |
| % | Percentage |
| ADA | American Dietetic Association |
| AIDS | Acquired immunodeficiency syndrome |
| AMF | Amylase-modified flour |
| AMS | Amylase-modified supplements/ Afr = Amilase-gewysigde supplemente |
| ARTs | Antiretroviral therapies |
| CDC | Centres for Disease Control and Prevention |
| CI | Confidence intervals |
| d4T | Stavudine |
| ddl | Didanosine |
| DNA | Deoxyribonucleic acid |
| EFV | Efavirenz |
| ELIZA | Enzyme linked immunosorbent assay |
| g | Grams |
| HAART | Highly active antiretroviral therapy |
| HIV | Human immunodeficiency virus |
| HIV-1 | Human immunodeficiency virus-1 |
| H/A | Height for age |
| H/W | Height for weight |
| Ig | Immunoglobulin |
| IgA | Immunoglobulin A |
| IgG | Immunoglobulin G |
| IL | Interleukin |
| kJ | Kilojoules |
| KS | Kaposi's sarcoma |
| LIP | Lymphocytic interstitial pneumonitis |
| mcg | Micrograms |
| mcg RE | Microgram Retinol Equivalents |

| | |
|---|---|
| mg | Milligrams |
| MIV | Menslike immuniteitsgebrekvirus |
| ml | Millilitre |
| mm$^3$ | Cubic millilitre |
| mOsm/l | Milliosmole per litre |
| mPa/s | Millipascal per second |
| NCHS | National Centre for Health Statistics |
| NFCS | National Food Consumption Survey |
| TNF | Tumour necrosis factor |
| NNRTI | Non-nucleoside analogue reverse transcriptase inhibitors |
| NRTI | Nucleoside analogue reverse transcriptase inhibitors |
| NVP | Nevirapine |
| PI | Protease Inhibitors |
| PEM | Protein-energy malnutrition |
| PEW | Proteïen-energie-wanvoeding |
| RDA | Recommended daily allowance |
| REE | Resting energy expenditure |
| RNA | Ribonucleic acid |
| SAVACG | South African Vitamin A Consultative Group |
| SD | Standard deviation from the median |
| TEE | Total energy expenditure |
| TNF | Tumour necrosis factor |
| UFS | University of the Free State |
| UNAIDS | Joint United Nations Programme on HIV/AIDS |
| UNICEF | United Nations Children fund |
| W/A | Weight for age |
| W/H | Weight for height |
| ZDV | Zidovudine |

# SUMMARY

Protein-energy malnutrition (PEM) is an important clinical manifestation of human immunodeficiency virus (HIV) infection in children and have immunosuppressive effects. Reduced energy and oral intake are the most prominent contributing factors leading to malnutrition. Several studies have proven that addition of amylase to bulky cereals decreases the viscosity of cereals and increases children's dietary intake. However, the impact of amylase modified supplements (AMS) on actual AMS consumption and energy intake from AMS by HIV-infected children is unknown.

The main objective of this study was to determine the actual supplement consumption and energy intake from a supplement by HIV-infected children. The study design was a double-blinded, randomized, clinical controlled prospective trial, and included 16 HIV-infected children resident in Lebone House.

Children were stratified according to baseline age, CD4+ counts and weight-for-age, and randomly placed into an experimental (E-) group and a control (C-) group. The E-group received an enzyme-modified, enriched maize supplement (E-supplement) and the C-group received an enriched maize supplement (C-supplement). The supplements were served as a breakfast replacement on 4 days per week, for a total period of 16 weeks. The actual supplement consumption was determined by subtracting the amount of leftover supplement from the amount of supplement served. The energy intake from the supplements was calculated by the Department of Biostatistics, University of Free State.

The actual supplement consumption was expressed as the mean amount of supplement consumed, the mean percentage of the served supplement consumed, and the percentage of days the participants consumed the entire supplement. The data on the actual supplement consumption demonstrated that the participants consumed large amounts (E-group 489g; C-group 490g) of supplements, which accounted to 98.1 percent and 98.6 percent of the E- and C-supplements served. The median of the percentage of times the E-group consumed the entire served supplement was 94.4 percent and 92.9 percent for

the C-group. No statistical significant difference was established between mean amount of supplement consumed (p=0.83), mean percentage of supplement consumed (p=0.67) and the percentage of times the entire served supplement was consumed (p=0.83). The actual supplement consumption was influenced by the viscosities of the supplements and cultural acceptability.

The mean energy intake from the supplement for both groups were high (E-group 2540.4 kJ; C-group 2553.2 kJ). The mean percentage of energy consumed from the supplement served was identical to the percentage of the served supplement consumed. No significant difference was observed for the energy intake between the two groups in terms of mean energy intake (p=0.67) and the mean percentage of energy consumed from the portion served (p=0.67). The energy intake of these HIV-infected children was increased with approximately 2000 kJ per day with the addition of a single portion of either supplement, even when the supplements were served as a replacement for their usual breakfast.

In conclusion, this study demonstrated that reducing the viscosity of the experimental supplement with amylase did not significantly increase the consumption or the energy intake. Both supplements were palatable and acceptable for these HIV-infected children and also increased the total daily energy intake of the children. Both supplements can therefore be used in the rehabilitation of HIV-infected children in South Africa.

Future research should evaluate whether the addition of amylase to an enriched soy-maize supplement would have a positive effect on the weight, immune status and health status of HIV-infected children in comparison to the control supplement without the added amylase. Future research should address the limitations mentioned in this study. Future application of the research if proven to have a significant benefit may include the use of the supplement as part of existing or new feeding schemes to improve the nutritional status of HIV-infected children.

**Key words:** HIV infection, HIV-infected children, stunting, wasting, amylase, germination, supplement consumption, energy intake.

# OPSOMMING

Proteïen-energie-wanvoeding (PEW) is 'n belangrike kliniese manifestasie van menslike immuniteitsgebrekvirus (MIV) in kinders en beskik oor immuunonderdrukkende effekte. Die oorsake van MIV-geassosieerde PEW is veelvoudig, maar verlaagde voeding- en energie-innames is die belangrikste faktore wat tot PEW kan aanleiding gee. Verskeie studies het bewys dat 'n verlaging in die viskositeit van graanvoedsel, kinders se dieetinnames kan verhoog. Die invloed van amilase-gewysigde supplemente (AMS) op die werklike supplementinname en energie-inname op MIV-geïnfekteerde kinders is nie bekend nie.

Die doelwit van die huidige studie was om die werklike supplement- en energie-inname vanaf die AMS op MIV-geïnfekteerde kinders te bepaal. In die studie is 'n dubbel-blinde gerandomiseerde, klinies-gekontroleerde, prospektiewe studieontwerp gevolg. Die studiedeelnemers was 16 MIV-geïnfekteerde kinders, wat in Lebone Tehuis woonagtig was.

Die studiedeelnemers is volgens hul basislyn-ouderdom, CD4+-tellings en massa-vir-ouderdom-status gestratifiseer en gerandomiseer in twee groepe, naamlik die eksperimentele (E-) en kontrole (C-) groep. Die E-groep het tydens die studie 'n amilase-gewysigde, verrykte mieliepap (E-supplement) ontvang, terwyl die kinders in die C-groep identiese mieliepap, maar sonder die ensiem amilase (C-supplement), ontvang het. Die E- en C-supplemente is as 'n ontbytplaasvervanger vier keer 'n week, oor 'n totale tydperk van 16 weke bedien. Die navorser het die hoeveelheid supplement wat bedien is, geweeg, asook die oorskiet. Die werklike supplementinname is bereken deur die oorskiet supplement af te trek van die hoeveelheid supplement wat voorgesit is. Die energie-inname van die supplemente is deur Departement Biostatistiek bereken.

Die werklike supplementinname is uitgedruk as gemiddelde supplementinname, gemiddelde persentasie van die ingeneemde supplement en persentasie van dae wat die deelnemers die volle porsie supplement ingeneem het. Die data vir die gemiddelde

supplement-inname dui aan dat die deelnemers groot hoeveelhede (E-groep 489g; C-groep 490.9g) supplemente ingeneem het. Die gemiddelde persentasies van die supplemente wat ingeneem is, was 98.1 persent (E-groep) en 98.6 persent (C-group). Die deelnemers het meestal (E-groep 94.4 persent; C-groep 92.9 persent) die volle supplementporsie wat bedien is, ingeneem. Geen betekenisvolle verskille het voorgekom tussen die E- en C-groep in terme van die gemiddelde supplementinname (p=0.83), gemiddelde persentasie van die supplementinname (p=0.67) en die persentasie van die aantal kere wat die volle supplementporsie ingeneem is (p=0.83). Die werklike supplementinname is beïnvloed deur die viskositeit van die supplemente en die kulturele aanvaarbaarheid.

Die gemiddelde energie-inname vanaf die supplemente was hoog (E-groep 2540.4kJ; C-groep 2553.2kJ) vir albei groepe. Die gemiddelde persentasie vir die energie-inname van die supplement was identies aan die persentasies van die supplementinname. Geen betekenisvolle verskille het tussen die E- en die C-groep voorgekom in terme van gemiddelde energie-inname (p=0.67) en gemiddelde persentasie van energie ingeneem van die porsie supplement bedien (p=0.67) nie.

Opsommend, in hierdie studie is gedemonstreer dat die verlaging in die viskositeit van die E-supplement met die ensiem, amilase, nie die supplement- of energie-inname van die supplement betekenisvol verhoog het nie. Beide die supplemente was smaaklik en kultureel aanvaarbaar vir hierdie Suid Afrikaanse MIV-geïnfekteerde kinders.

Toekomstige navorsing behoort te bepaal of die byvoeging van amilase tot verrykte mieliepap 'n positiewe effek sal hê op die massa, immuun- en gesondheidstatus van MIV-geïnfekteerde kinders in vergelyking met 'n kontrole pap, sonder bygevoegde amilase. Die uitgewysde beperkings van hierdie studie sal in hieropvolgende studies in ag geneem moet word. Toekomstige toepassing van hierdie projek, indien daar voordele met die gebruik van amilase ontdek word, sluit die insluiting van die E-supplement by bestaande of nuwe voedingsprogramme in – om sodoende die voedingstatus van MIV-geïnfekteerde kinders te verbeter.

**Trefwoorde:** MIV-infeksie, MIV-geïnfekteerde kinders, groeiinkorting, wegkwyning, amilase, ontkieming, supplement-inname, energie-inname.

# CHAPTER 1

# INTRODUCTION

## 1.1    PROJECT OUTLINE

This study formed part of a larger study in which three researchers participated. The first researcher determined the impact of the enzyme-modified, enriched maize-based supplement (experimental supplement) on the weight, immune and health status of children infected with human immunodeficiency virus (HIV). The second researcher determined the impact of the experimental product (nutritional analysis indicated in Table 3.2, Chapter 3) on the anthropometric nutritional status of the HIV-infected children. This third study focuses on the supplement consumption and energy intake of HIV-infected children. A flow diagram of the methodological procedures of the main research project is outlined in Figure 3.1 (Chapter 3).

## 1.2    PROBLEM STATEMENT

In 2004 the total number of people living with HIV rose to reach its highest level ever. In that year alone, approximately 2000 children under the age of 15 years were newly infected with HIV per day, reaching the sum of approximately 640 000 children. An estimated 2.2 million children of all ages were living with HIV at the end of 2004, while 510 000 children died due to acquired immunodeficiency syndrome (AIDS) in 2004 (Joint United Nations Programme on HIV/AIDS [UNAIDS], 2004).

Sub-Saharan Africa remains the worst affected region in the world (UNAIDS, 2004). According to Noah (2003), the word *epidemic* seems inadequate to describe the spread of the HIV virus in sub-Saharan Africa, as the HIV *pandemic* rivals the worst of history's disease outbreaks. Logie (1999) speculates that the life expectancy for sub-Saharan Africans were likely to fall from 63 years in 1998 to 45 years in 2005.

In developed countries, paediatric HIV infection is on the verge of being eliminated. In sub-Saharan Africa, however, HIV infection has become a common cause of childhood mortality (Stover and Way, 1998). Sub-Saharan Africa has just over ten percent of the world's population, but is home to more than 60 percent of all people living with HIV, with 1.9 million HIV-infected children under 15 years at the end of 2004 (UNAIDS, 2004). In addition, large numbers of children have become orphaned. At the end of 2000, UNAIDS estimates that there were over 12 million orphans in Africa who lost their parents to AIDS (cited by Cant *et al.*, 2003, p. 1295).

Southern Africa remains the worst affected sub-region in the world, with South Africa having the highest number of people living with HIV in the world (Cant *et al.*, 2003, p. 1295; UNAIDS, 2004). In 1995, 47 percent of black women were found to be HIV infected at Hlabisa Hospital, Kwa-Zulu Natal (Walker, 2001). In 2000, Gottlieb (2000) speculated that half of black South African children were likely to die from HIV infection. In 2001, the South African Government released its annual figures on HIV/AIDS, which showed a continued increase in the numbers of people contracting HIV. In 1999, about 4.2 million South Africans were infected with HIV, compared with 4.7 million in 2001 (Sidley, 2001). The earlier surveys showed that until 1998 South Africa had one of the fastest expanding epidemics in the world. The Department of Health's National HIV and Syphilis Sero-prevalence Survey of 2003, however, indicated that the trend has since changed, so that the level of HIV prevalence is now growing more slowly (Noble *et al.*, 2005). Despite this trend, at the end of 2004 South Africa still had the highest number of people living with HIV in the world (UNAIDS, 2004).

The Nelson Mandela Study (2002, as cited by Noble *et al.*, 2005) estimated that 11.4 percent of all South Africans over the age of two years were HIV positive in 2002. Among those between 15 and 49 years of age, the estimated prevalence rate was 15.6 percent in 2002 (Noble *et al.*, 2005). The South African Department of Health Study of 2003 estimated that 5.6 million South Africans were HIV infected at the end of 2003, of whom 55 percent were female and 96,228 were babies (Noble *et al.*, 2005). UNAIDS (2004) estimated that 5.3 million South Africans were infected with HIV at the end of

2004, 2.9 million of them women. During an epidemiological study performed in Mangaung, Bloemfontein, the HIV prevalence for women between 25 to 34 years, and women between 35 to 44 years, was 61 and 38 percent, respectively (Walsh *et al.*, 2004; Walsh *et al.*, 2002). In 2003, the estimated HIV prevalence among antenatal clinic attendees in the Free State was 30.1 percent (Noble *et al.*, 2005). Dannhauser *et al.* (2002) add that a significant number of persons infected with HIV in the Free State have a high nutritional risk, since the staple food of the population is primarily maize and the HIV-infected persons often are of a lower socio-economic group.

Prior to HIV awareness, South Africa already had a high incidence of protein-energy malnutrition (PEM). The South African Vitamin A Study, performed on children between 6 and 71 months old, indicated that almost one in four children was stunted and one in ten was underweight (South African Vitamin A Consultative Group [SAVACG], 1996). The National Food Consumption Survey (NFCS) performed on children between the ages of one and nine years old indicated that one out of ten children was underweight and one in five was stunted (Labadarios and Nel, 2000, p. 183). Secondary analysis of the height measurements of the children in the National Food Consumption Survey showed the national prevalence of stunting was estimated at 19.3 percent (Steyn *et al.*, 2005).

HIV-infected children admitted to Chris Hani Baragwanath Hospital, from October, 1996 to December, 1997, weighed significantly less for age than the HIV negative children. Eighty one percent of the HIV-infected children weighed less than 80 percent of the expected weight-for-age, compared with 39.5 percent of the seronegative children (Johnson *et al.*, 2000). A number of studies (Steenkamp *et al.*, 2004;  Eley *et al.*, 2002) done amongst HIV-infected preschool children (between the ages of 18 and 72 months) in South Africa, showed figures of 28 to 50.9 percent for the prevalence of underweight and 58 to 58.8 percent for stunting. These statistics were much higher than the average percentages indicated in both the South African Vitamin A Study (SAVACG, 1996) and the NFCS of 1999 (Labadarios and Nel, 2000, p. 183) done on children.

Preschool children (younger than 72 months) living in rural areas of Bloemfontein, generally have poor nutritional status, and require urgent intervention (Dannhauser *et al.*, 1996; Dannhauser *et al.*, 2000). Since it has been shown that the prevalence of PEM is even higher amongst HIV-infected children compared to seronegative children, it can be assumed that HIV infection could further worsen the development of PEM amongst already undernourished children.

HIV-infected patients may be at nutritional risk at any point in their illness (Nerad *et al.*, 2003). Growth failure, undernutrition (Miller *et al.*, 1993) and wasting (Macallen, 1999[b]) are among the most frequent complications seen in HIV-infected children (Sun and Sangweni, 1997). According to Grinspoon and Mulligan (2003) weight loss and wasting remain significant clinical problems, even in the era of potent antiretroviral therapy (ART). For many HIV-infected children, death seems to be determined more by the individual's nutritional status than by any particular opportunistic infection (Arpadi *et al.*, 2000; Sun and Sangweni, 1997).

AIDS-associated wasting has been seen as the result of:
- poor oral intake and appetite (Miller *et al.*, 1993), including poor energy, protein, carbohydrate and vitamin intake (Sun and Sangweni, 1997);
- gastrointestinal malabsorption (Miller *et al.*, 1993); and
- metabolic effects of infection and inflammation, induced by cytokines (Fields-Gardner and Ayoob, 2000; Macallen, 1999[b]) and possibly leading to excessive energy expenditure (Fenton and Silverman, 2004, p. 1044).

The deleterious effects that HIV associated wasting has on an infected individual include:
- higher demands on the bodies and immune system  because of PEM (Sun and Sangweni, 1997);
- immune dysfunction and greater susceptibility to disease progression with PEM as cofactor (Miller *et al.*, 1993); and
- vitamin and mineral deficiencies resulting in immune function abnormalities (Baum *et al.*, 1995; Sun and Sangweni, 1997).

Reduced oral intake is the most prominent factor leading to wasting and malnutrition in HIV-infected children (ADA, 2000, p. 432; Cant *et al.*, 2003, p. 1305). Children with HIV infection suffer from poor oral intake, poor appetite (Miller *et al.*, 1993), odynophagia, dysphagia, and abdominal pain (Miller, 1996). Diarrhoea is the most common gastrointestinal symptom in patients with HIV disease (ADA, 2000, p. 431) and during episodes of diarrhoea, the energy intake from food in children is further decreased (Mitra *et al.*, 1995).

Dietary bulk and high viscosity are factors that reduce oral intake (Rahman *et al.*, 1995). A starch-based staple food such as maize has a low energy density (Den Besten *et al.*, 1998; Michaelsen and Friis, 1998; Mitra *et al.*, 1995) and a high viscosity. According to Bennett *et al.* (1999) viscosity may limit oral intake when the consistency of the diet exceeds a threshold above which children can no longer readily chew and swallow the food. Young children find it difficult to eat staples with high viscosity, even when they are not sick (Den Besten *et al.*, 1998; Mitra *et al.*, 1995).

The negative impact of dietary bulk on oral intake can also be overcome by liquefying an energy-dense, thick porridge with amylase (Gopaldas and John, 1992; Rahman *et al.*, 1997). According to Mahalanabis *et al.* (1993) and Rahman *et al.* (1997) amylase is an enzyme that hydrolyses starch and liquefies a sticky semi-solid porridge by reducing the viscosity. Thus, the amylase decreases the consistency of the porridge to a level where the child can readily chew and swallow the porridge, thereby increasing the intake of the porridge.

It has been shown that energy intake is substantially increased in infants and young children when they are fed an energy-dense diet, liquefied with amylase-modified flour (AMF), and feeding such an energy-dense high osmolar diet did not have any adverse effects (Bennett *et al.*, 1999; Den Besten *et al.*, 1998; Gopaldas and John, 1992; John and Gopaldas, 1993; Moursi *et al.*, 2003).

Since it has been shown that high viscosity can reduce oral intake in healthy children, it can be assumed that high viscosity porridges could further decrease oral intake amongst already malnourished and anorectic HIV-infected children. Thus, it is possible that the addition of amylase to high viscosity porridge could decrease the viscosity of the porridge to a level where an HIV-infected child can more readily chew and swallow the porridge, thereby increasing the oral intake and, possibly, energy intake.

However, no studies have been done on HIV-infected children to evaluate the impact of amylase on the actual consumption of amylase-modified supplements (AMS) and the energy intake from the AMS.

## 1.3    OBJECTIVES

The main aim of this study is therefore to determine the actual consumption and energy intake from supplements, by HIV-infected children.

The objectives of the study were to determine:
- the actual consumption of the experimental and control supplement over a period of 16 weeks;
- the energy intake from the supplement over the same period;
- differences in the actual supplement consumption and energy intake resulting from the supplements given to the participants in the experimental and control groups.

## 1.4.    STRUCTURE OF SCRIPT

The script is divided into six chapters. The first chapter gives a brief overview and introduction to the dissertation. The second chapter reviews the most recent, HIV related literature. The third chapter discusses the methodology of the research project. The fourth chapter presents the research results, while the fifth chapter is devoted to the discussion and recommendations.

# CHAPTER 2
# LITERATURE REVIEW

## 2.1    INTRODUCTION

HIV infection is characterized by progressive immunologic deterioration and associated opportunistic infections and malignancies (Beers and Berkow, 1999, p. 2345; ADA, 2000, p. 429). Throughout the course of HIV disease, HIV-related problems may negatively affect nutritional status, causing malnutrition (ADA, 2000, p. 429). Malnutrition is among the most frequent complications seen in children with HIV/AIDS (Fenton and Silverman, 2004, p. 1044). Reduced energy or oral intake is the most prominent contributing factor leading to the development of malnutrition and wasting in HIV-infected children (Cant *et al.*, 2003, p. 1305; Macallen, 1999[b]).

The provision of adequate nutrition may help to delay the progression of HIV to AIDS. Early nutrition intervention in patients with HIV infection may help to improve clinical, functional, and psychological well-being (ADA, 2000, p. 431).

In this literature, an overview on HIV-infection in children is highlighted, together with the relationship between HIV-infection and malnutrition. The impact of nutrition intervention in HIV-infected children is described with emphasis on the role of amylase.

## 2.2    HIV INFECTION IN CHILDREN

The transmission, pathogenesis, diagnosis and manifestations, and the medical treatment of HIV-infected children are indicated in the discussion below.

### 2.2.1   Transmission

Vertical transmission now accounts for almost all new cases of HIV in pre-adolescent children. The infection risk for an infant born to an HIV-positive mother who did not

receive antiretroviral therapy (ART) during pregnancy is estimated at 13 to 39 percent. The risk for HIV-transmission may be higher for infants born to mothers who seroconvert during pregnancy and those with advanced disease (Beers and Berkow, 1999, p. 2345; Stiehm, 1996). Adverse birth events, particularly premature rupture of membranes, are associated with risk of HIV-infection (Goedert and Coté, 1994). The risk of HIV transmission can be reduced with caesarean section and zidovudine therapy (Beers and Berkow, 1999, p. 2345; Goedert and Coté, 1994).

Breast milk of HIV-infected mothers contains HIV as well as protective ingredients, such as HIV antibodies and glycoprotein that inhibits the binding of the virus to CD4+ T-lymphocytes. Despite the presence of these protective factors, a number of cases of transmission of HIV by breastfeeding have been documented (Stiehm, 1996). Risk of transmission by breastfeeding may be increased in mothers with high plasma viral concentrations (Beers and Berkow, 1999, p. 2346).

### 2.2.2   Pathogenesis

HIV is a retrovirus that predominately infects a subset of T-lymphocytes (Beers and Berkow, 1999, p. 1314; Cant *et al.*, 2003, p. 1300; Fenton and Silverman, 2004, p. 1030), carrying the surface molecule CD4, which binds the glycoprotein on the envelope of HIV called gp120 (Cant *et al.* 2003, p. 1300). Beers and Berkow (1999, p. 1314) defines the CD4+ T-lymphocytes phenotypically by the CD4 transmembrane glycoprotein, and functionally as helper/inducer cells.

HIV requires co-receptors to enter cells. Two of the important co-receptors are CCR5 and CXCR4. These co-receptors function as receptors for chemokines that orchestrate the migration and differentiation of leukocytes during immune responses (Cant *et al.*, 2003, p. 1300). Following the fusion of HIV into CD4 and other cells, viral core material enters the host cells and the generic material encoded in ribonucleic acid (RNA) is converted to deoxyribonucleic acid (DNA) by reverse transcriptase. This DNA provirus is then integrated into the host genome (Cant *et al.*, 2003, p. 1300). The proviral DNA is both

transcribed to RNA and translated to proteins to produce hundreds of copies of the infectious virus. Critical to the final step in the life cycle of HIV is another enzyme, HIV protease. HIV protease converts immature, non-infectious HIV to its infectious form by splitting crucial proteins so they can rearrange within the virus after it has budded from an infected human cell (Beers and Berkow, 1999, p. 1314).

The immunologic dysfunctions in AIDS appear to be explained by loss of the helper function of CD4+ lymphocytes – which is critical to cell-mediated immunity (Beers and Berkow, 1999, p. 1314; Cant *et al.*, 2003, p. 1300) and antibody-immune response (ADA, 2000, p. 429). The pattern of loss of CD4+ T-lymphocytes proceeds in three phases and at rates that vary from patient to patient. Within the first months of infection, the number of circulating CD4+ cells drop rapidly. A prolonged period of slower decline may be followed by another more rapid decline in the one to two year period before AIDS develops (Beers and Berkow, 1999, p. 1315).

The plasma level of viral RNA (viral load) rises over the first weeks after primary infection around the time of birth, and stays high throughout infancy. It then continues to fall in the absence of therapy over the next three to five years, to reach a set point which is much later after primary infection than in adults (set point at about six months). Infants have a highly active thymus which may replenish CD4 cells destroyed by the virus. As viremia starts to decline, HIV antibody production increases and forms the principal way of diagnosis in adults and older children, as antibodies usually persist until death (Cant *et al.*, 2003, p. 1300).

Suppressor/ cytotoxic CD8+ lymphocytes appear to be functionally normal and more in number with HIV infection, which may contribute further to immunosuppression and results in reduction of the CD4:CD8 ratio (normally ~ 2:1) to a ratio of less than one (Beers and Berkow, 1999, p. 1314).

Hyperplasia of B-lymphocytes (antibody-producing) in lymph nodes causes lymphadenopathy and increased secretion of antibodies, leading to hyperglobulinemia.

Production of antibodies to previous encountered antigens persists; however, response to new antigens is defective and sometimes absent. Thus, total antibody levels (especially IgG and IgA) may be elevated (Beers and Berkow, 1999, p. 1315) but leads to increased susceptibility to bacterial infections (Cant *et al.*, 2003, p. 1300).

### 2.2.3 Diagnosis and manifestations

HIV is usually diagnosed with serum antibody tests (enzyme linked immunosorbent assay [ELISA] and confirmatory Western Blot), except in children younger than 18 months of age. Children younger than 18 months may have passively acquired maternal HIV antibodies (Beers and Berkow, 1999, p. 2349) through transplacental transfer (Cant *et al.*, 2003, p. 1301). Thus, all babies born to HIV-infected women will have antibodies at birth which take a median of 10 months and a maximum of 18 months to clear. Therefore, other direct techniques are required to diagnose HIV in young infants (Cant *et al.*, 2003, p. 1301).

HIV-infected babies appear normal at birth with birth weight generally in the normal range (Cant *et al.*, 2003, p. 1302). Although the median age symptom onset is estimated to be three years, increasing numbers of children remain asymptomatic for more than five years (Beers and Berkow, 1999, p. 2346). As in adults, HIV may present with a spectrum of in children (Beers and Berkow, 1999, p. 2346; Cant *et al.*, 2003, p. 1303), but the symptoms and signs are frequently common in general paediatrics and are non-specific (Cant *et al.*, 2003, p. 1303).

The most common manifestations of HIV infection in children include generalized lymphadenopathy, hepatomegaly, splenomegaly, failure to thrive, oral candidiasis, recurrent diarrhoea, parotitis, fever, recurrent otitis (Cant *et al.*, 2003, p. 1302), cardiomyopathy, hepatitis, nephropathy, central nervous system disease, lymphoid interstitial pneumonitis, opportunistic infections, and malignancies (Beers and Berkow, 1999, p. 2346).

Important differences between adult and paediatric manifestations are (Beers and Berkow, 1999, p. 2346; Cant *et al.*, 2003, p. 1303):

- faster rate of disease progression, especially in infants;
- lymphocytic interstitial pneumonitis (LIP) and parotitis common;
- more bacterial infections;
- differently presented encephalopathy;
- growth failure and wasting;
- rare prevalence of Kaposi's sarcoma (KS) outside endemic areas;
- different immunology;
- higher numbers and more variation of CD4 cells;
- decline to adult values in mid-childhood;
- less variable CD4 percentage; and
- different pattern of HIV RNA –decline of viral load up to five years.

### 2.2.4 Medical treatment

A detailed description of the many aspects of ARTs and highly active antiretroviral therapy (HAART) is beyond the scope of this script. However, a short description of the different ARTs currently used in paediatrics will be given. Table 2.1 shows the different drugs used in medical therapy. There are 16 drugs belonging to three different classes now available for the treatment of HIV infection in adults (Cant *et al.*, 2003, p. 1305).

**Table 2.1** Antiretroviral therapies (Cant *et al.*, 2003, p. 1305)

| Nucleoside Analogue Reverse Transcriptase Inhibitors (NRTIs) | Nucleotide Analogue Reverse Transcriptase Inhibitors | Non-Nucleoside Analogue reverse Transcriptase Inhibitors (NNRTIs) | Protease Inhibitors |
|---|---|---|---|
| Zidovudine (ZDV)*<br>Didanosine (ddl)*<br>Dideoxycytidine*<br>Lamivudine (3TC)*<br>Stavudine (d4T)*<br>Abacavir *♦ | Tenofovir ♦†<br><br>**Entry inhibitors**<br><br>T-20 ♦† (injection) | Nevirapine (NVP)*<br>Delaviridine ♦†<br>Efavirenz (EFV)†* | Ritonavir†*<br>Nelfinavir †*<br>Amprenavir ♦†*<br>Lopinavir ♦†*<br>Indinavir ♦† |

\*      Paediatric formulation

†      Inadequate pharmacokinetic studies in infants

♦      Unlicensed in Europe

Combination ART has turned HIV into a treatable chronic disease of childhood (Cant *et al.*, 2003, p. 1307; Hammami *et al.*, 2004). Eradication of HIV is not possible with current drugs, not even after the virus has been suppressed to below the level of detectability in plasma. Most children on HAART remain clinically very well, thriving normally and asymptomatic. However, the complexity of life time administration should not be underestimated (Cant *et al.*, 2003, p. 1305).

Patients must adhere to daily and often very complicated drug schedules, which may have some specific meal and food requirements. Intake must be more than 95 percent adherent to drug schedules for medications to work well. Late, missed, or non-meal coordinated medications may increase risks for sub-optimal dosing, viral breakthrough, and the development of drug-resistant strains of HIV (Fenton and Silverman, 2004, p. 1042). It has become very clear from paediatric studies that adherence to a complicated drug schedule is one of the principal determinants of both the degree and duration of virological suppression (Cant *et al.*, 2003, p. 1307). Hammami *et al.* (2004) conducted a

qualitative study to investigate factors that influence adherence to HAART in a paediatric population. The data indicate that coping with HIV and the process of establishing good adherence may be interrelated.

HIV-infected children who are treated with mono- or dual nucleoside analogue transcriptase inhibitor (NRTI) therapy show a temporary increase in weight and linear growth rate. Height and growth rate are favourably influenced in children in whom HAART leads to a reduction of the viral load and an increase in the CD4+ counts (Verweel *et al.*, 2002). Rutstein *et al.* (1997) presented evidence that combination therapy regimes, including protease inhibitor (PI), will lead to significant short-term virologic and immune improvement in HIV-infected children. Melvin *et al.* (2001) explain that treatment with combination regimes including protease inhibitors, frequently results in the suppression of HIV-1 plasma RNA to below the level of detection. PI therapy in children has a positive effect on several growth parameters, including weight, weight for height and muscle mass (Miller *et al.*, 2001). According to Buchacz *et al.* (2001) the use of protease inhibitor-containing ARTs was associated with only small annual increments in height and growth. Rutstein *et al.* (1997) suggested that long-term follow up studies should examine the overall result of combination therapy regimes, including PI, on the quality of life or on survival. As people were monitored on these regimes for longer periods, several potentially significant side-effects have emerged (Melvin *et al.*, 2001).

A new syndrome, lipodystrophy syndrome, has recently emerged in adults receiving ARTs (Arpadi *et al.*, 1999; Melvin *et al.*, 2001; Fenton and Silverman, 2004, p. 1042). Lypodystrophy syndrome includes body fat accumulation and fat atrophy, hyperlipidemia, insulin resistance or glucose intolerance, lactic acidosis, osteopenia and osteoporosis (Cant *et al.*, 2003, p. 1307), avascular necrosis and bone fracture, and mitochondrial toxicity (Fenton and Silverman, 2004, p. 1042). The development of hyperglycemia and lipid abnormalities may increase the risk of diabetes, heart disease and stroke (Nerad *et al.*, 2003). Although initial reports implicate PI in the development of lypodystrophy, more recent studies suggest that nucleoside analogues may play a role (Brambilla *et al.*, 2001; Carr *et al.*, 2000).

Limited data are available on the long-term use of ARTs in the treatment of HIV-infected children. However, increased central fat and peripheral lipoatrophy are distinctive features of all HAART-treated children. But the impact of the long-term use of ARTs on HIV-infected children's lipid profiles and cardiovascular risks is unknown. As toxicity with long-term continuous ART becomes more apparent, new strategies including structured treatment interruptions and immune therapies (Cant *et al.*, 2003, p. 1308) and nutrition intervention (ADA *et al.*, 2000, p. 432) need evaluation in children.

## 2.3 MALNUTRITION IN HIV-INFECTED CHILDREN

The complex relationship between malnutrition, the immune system and HIV is described, and the pathogenesis of malnutrition in HIV-infected children is highlighted in the sections that follow.

### 2.3.1 Relationship between HIV and malnutrition

The effects of HIV infection on and its complications for nutritional status and the effect of nutritional status on HIV have been thoroughly explored (Fenton and Silverman, 2004, p. 1028; Fields-Gardner and Ayoob, 2000). It has been well established that deficiencies and sometimes excesses of nutrients adversely affect immune and other normal body processes (Fields-Gardner and Ayoob, 2000).

PEM can have adverse, even devastating effects on the antigen-specific arms of the immune system, as well as on many more generalized mechanisms for host defence (Beisel, 1996; Sun and Sangweni, 1997). Generalized PEM causes widespread atrophy of lymphoid tissues, especially in children. The thymus, spleen, tonsils and lymph nodes are all affected, with atrophy being greatest in the T-lymphocyte areas of these tissues (Beisel, 1996).

The immune system, malnutrition, and infectious diseases are locked into a complex three-way relationship. Infectious diseases give rise to serious problems of malnutrition

in children; malnutrition, in turn, leads to a variety of immune system dysfunctions; and ineffective immunity, with its accompanying impairment of body defence mechanisms, allows infectious diseases to flourish. These closely-linked events can initiate a downward spiral or a vicious cycle that leads inexorably to death (Beisel, 1996; Macallen, 1999[a]).

PEM causes a marked repression of cell-mediated immunity and the function of T-lymphocytes. Malnourished children show a decrease or reversal of the T-helper/suppressor cell ratio, and loss of the ability of killer lymphocytes to recognize and destroy foreign tissues. In contrast, B-lymphocyte numbers and function generally appear to be maintained. While existing antibody production is conserved or even increased during generalized malnutrition, new primary antibody responses to T-cell dependent antigens and antibody affinity are impaired (Beisel, 1996).

Deficiencies in any of a large number of single nutrients may produce dysfunctions in the immune system and other host-defensive mechanisms. Importantly, single nutrient deficiencies often co-exist with the generalized nutritional problems included under PEM. Clinically, the most important of the single nutrient deficiencies, in terms of their immunological effects in malnourished children, are vitamins A and C, and the trace minerals, iron and zinc (Beisel, 1996). For South African children as a whole (between one and nine years), the dietary intake of the following nutrients was less than 67 percent of the recommended daily allowance (RDA's): energy, vitamin A, vitamin D, vitamin C, vitamin E, riboflavin, niacin, vitamin B6, iron, zinc, selenium and calcium (MacIntyre and Labadarios, 2000, p. 352).

Malnutrition and involuntary weight loss are among the most frequent complications seen in children with HIV/AIDS (ADA, 2000, p. 429; Arpadi *et al.*, 2000; Fenton and Silverman, 2004, p. 1044; Hirschfeld, 1996; Johann-Liang *et al.*, 2000; Sun and Sangweni, 1997). HIV targets the immune system, making an infected person susceptible to infection and neoplasm because of an impaired ability to mount an adequate immune

response. Malnutrition and its complications further render an HIV-infected person susceptible to opportunistic infection (Fields-Gardner and Ayoob, 2000).

Direct and indirect nutritional factors mechanisms are responsible for the impact of nutrition on HIV. Directly, nutritional factors are required for specific immune-cell triggering, interaction, and expression. Indirectly, nutritional factors are essential for DNA and protein synthesis and for the physiologic integrity of cell tissues and organ systems, including lymphoid tissues. Malnutrition may contribute to the frequency and severity of infection seen in AIDS by compromising immune function (Fenton and Silverman, 2004, p. 1045). Malnutrition can result in premature death among HIV-infected children, especially children from socio-economic disadvantaged families (Sun and Sangweni, 1997).

Medical nutrition therapy should be an integral part of ongoing health care of people with HIV to address a multiple of factors that can contribute to health decline (Fields-Gardner and Ayoob, 2000). Nutritional supplements offer the potential to moderate immuno-suppression and progression of AIDS to death (Watson, 1992). One area where nutrition intervention was shown to have clear benefits was in those individuals with severe malnutrition and wasting, in whom the provision of adequate nutrition had a dramatic effect on survival (Macallen, 1999[a]). However, much more information on nutritional supplementation to enhance immune responses in human subjects, and especially children, is needed.

### 2.3.2 Pathogenesis of wasting and stunting in HIV-infected children

Reduced levels of growth have been described in HIV-infected children (Carey *et al.*, 1998; Moye *et al.*, 1996). HIV-infected children may suffer from growth failure, developmental delay and malnutrition from as early as four months of age (Fields-Gardner and Ayoob, 2000). Wasting and stunting are frequent and complicated consequence of HIV infection (Alfaro *et al.*, 1995; Heller *et al.*, 2000) and contributes to

morbidity and mortality (ADA, 2000, p. 429; Arpadi *et al.*, 2000; Fenton and Silverman, 2004, p. 1044; Hirschfeld, 1996; Johann-Liang *et al.*, 2000; Sun and Sangweni, 1997).

According to Cant *et al.* (2003, p. 1302) failure to thrive and poor growth have prognostic significance, because growth faltering is an indication of increasing immune deficiency, whereas weight loss is characteristic of disease progression (Woods *et al.*, 2002). Acute wasting is associated with secondary infections, and chronic wasting is associated with gastrointestinal disease (Macallen, 1999[b]). Henderson *et al.* (1994) add that poor growth has been shown to precede a decline in CD4 count and the development of opportunistic infection. According to Arpadi *et al.* (2000) growth failure in HIV-infected children is related to active viral replication as reflected in the circulating viral load.

The causes of malnutrition are multifactorial (ADA, 2000, p. 432; Cant *et al.,* 2003, p. 1305; Fenton and Silverman, 2004, p. 1051; Henderson *et al.*, 1998; Macallen, 1999[b]; Miller, 1996). Problems leading to malnutrition may involve inadequate ingestion, absorption, digestion, metabolism and use of nutrients (Fenton and Silverman, 2004, p. 1044). According to Miller (1996) only a limited number of studies (especially on children) have identified risk factors of malnutrition in HIV infection. These factors include fever, diarrhoea, acute infection and anorexia. Three potential mechanisms for weight loss include inadequate oral intake, gastrointestinal malabsorption and abnormal energy metabolism.

## 2.3.2.1 Energy intake

Reduced energy or oral intake is the most prominent contributing factor leading to the development of malnutrition and wasting (Cant *et al.*, 2003, p. 1305; ADA, 2000, p. 432; Johann-Liang *et al.*, 2000; Macallen, 1999 [b]; Miller *et al.*, 1993).

AIDS wasting has been associated with poor oral intake and loss of appetite (Miller, 1993), including poor energy, protein, carbohydrate and vitamin intake (Sun and Sangweni, 1997). A variety of potential factors may lead to insufficient energy intake,

such as lesions in the gastrointestinal tract, pancreatic and biliary disease, cytokines and encephalopathy (Miller, 1996).

Decreased oral intake is very common and can result from anorexia secondary to medications, depression, oral and oesophageal infection, symptoms such as nausea, vomiting, diarrhoea, dyspnoea, neurological disease (Fenton and Silverman, 2004, p. 1044), abdominal discomfort, dementia, and fatigue. Inadequate finance and lack of access to food also lead to decreased oral intake (ADA, 2000, p. 432).

Inflammation and ulcers of the upper gastrointestinal tract are also conditions that can lead to anorexia owing to odynophagia, dysphagia, or abdominal pain that is associated with eating. These lesions may be due to infectious agents (Miller, 1996). The T- and B-cell abnormalities resulting from HIV infection result in increased susceptibility to a wide range of organisms (Cant *et al.*, 2003, p. 1304). Opportunistic infections are common among HIV-infected children (Cant *et al.*, 2003, p. 1304), and are often the cause of anorexia and weight loss (ADA, 2000, p. 430; Fenton and Silverman, 2004, p. 1034).

*Candida albicans* and the herpes simplex virus cause sores of the mouth, pain or difficulty with eating and swallowing, and also reduce oral intake (ADA, 2000, p. 430; Fenton and Silverman, 2004, p. 1052; Miller, 1996). Oral ulcers caused by viral agents or idiopathic oral ulcers are common among HIV-infected children (Miller, 1996). Cytomegalovirus causes ulcerative lesions in the entire gastrointestinal tract. The manifestations linked to cytomegalovirus include oesophagitis, gastritis, enteritis, colitis, watery or bloody diarrhoea, and organ perforation (ADA, 2000, p. 430).

Malignancies are relatively uncommon in HIV-infected children (Beers and Berkow, 1999, p. 2346). Kaposi's sarcoma is a malignant disease of the peripheral blood mononuclear cells that manifests as purple nodules on the skin, mucous membranes, and lymph nodes or throughout the gastrointestinal tract (Fenton and Silverman, 2004, p. 1036). Kaposi's sarcoma lesions in the oral cavity or oesophagus may cause pain with chewing and swallowing, and lesions in the intestinal tract have been implicated in

diarrhoea and intestinal obstruction (ADA, 2000, p. 430; Fenton and Silverman, 2004, p. 1036).

Pancreatic and biliary tract disease can cause vomiting and abdominal pain in HIV-infected children, leading to poor oral intake. Pancreatic disease has been linked to medications and opportunistic infections, especially with cytomegalovirus and *mycobacterium avium* (ADA, 2000, p. 430; Miller, 1996). Biliary tract disease with sclerosing cholangitis and papillary stenosis has been linked to cryprosporium, cytomegalovirus and microsporidia (Miller, 1996).

Primary anorexia may also contribute to inadequate oral intake. It is postulated that increased cytokine production (tumour necrosis factor [TNF], interferon-gamma and interleukin-1 [IL-1] and interleukin-6 [IL-6]) may be associated with anorexia. TNF also causes delayed gastric emptying which can increase anorexia as well. Currently, the scientific data that implicate these cytokines as mediators of anorexia are controversial (Hellerstein *et al.*, 1993; Miller, 1996).

Immediately after infection, HIV enters the brain and may result in encephalopathy or AIDS dementia (Fenton and Silverman, 2004, p. 1036). HIV encephalopathy is seen most frequently in the subgroup of children with rapid disease progression. The most common neurological manifestations are hypertonic diplegia, developmental delays (particularly affecting motor skills) or acquired microcephaly (Cant *et al.*, 2003, p. 1304), and decreased sensory perception (Fenton and Silverman, 2004, p. 1053).

HIV encephalopathy may result in the inability to consume enough calories to sustain growth. Oral administration of feedings under this condition may be dangerous owing to the high risk of aspiration in neurologically compromised children (Fenton and Silverman, 2004, p. 1053; Miller, 1996).

The decreased oral intake of HIV-infected children can finally, result from anorexia secondary to medication (Fenton and Silverman, 2004, p. 1044). Many medications

prescribed for HIV-infected children result in gastric irritation, vomiting, and nausea (Miller, 1996).

## 2.3.2.2 Diarrhoea and malabsorption

Gastrointestinal disturbances related to recurrent opportunistic infections and malabsorption, including carbohydrate malabsorption, has been implicated in the poor growth of HIV-infected children (Johann-Liang *et al.*, 2000).

Malabsorption is often suspected in the event of loose stools, diarrhoea, or vomiting. Malabsorption can be caused by medications, HIV infection, opportunistic infections or a developed intolerance to lactose, fat, or gluten (Fenton and Silverman, 2004, p. 1044). Malabsorption can result from villous atrophy associated with malnutrition, fluid, albumin shifts that can alter osmotic pressure in already compromised gut tissue, and pancreas insufficiency (ADA, 2000, p. 432). Poor absorption may lead to a deficiency of vitamins A, C, $B_6$, $B_{12}$ and folate and the minerals iron, selenium, and zinc (Fenton and Silverman, 2004, p. 1051).

Diarrhoea is the most common gastrointestinal symptom in patients with HIV. Multiple aetiologies can be responsible for diarrhoea in HIV patients, including a wide variety of protozoal, viral, and bacterial pathogens, including cytomegalovirus, *giardia lamvlia, Isospora belli*, *Camphylobacter jejuni, Clostridium difficile,* mycobacterium complex, salmonellae and shigella (ADA, 2000, p. 430-431).

The HIV virus can disrupt the intestinal mucosa, causing motility disturbances, which can also result in diarrhoea. Other aetiologies of diarrhoea can include idiopathic colitis and small bowel overgrowth. Small bowel bacterial overgrowth results in a syndrome of diarrhoea and malabsorption of fat, vitamin $B_{12}$, and carbohydrate (ADA, 2000, p. 432).

## 2.3.2.3 Metabolism

Abnormalities in metabolism have been seen in HIV disease (ADA, 2000, p. 433, Alfaro *et al.*, 1995; Crenn *et al.*, 2004; Jahoor *et al.*, 2003; Macallen, 1999[b]). According to Miller (1996) increased energy metabolism of HIV-infected children can contribute to malnutrition by increasing the child's energy demands to maintain weight and sustain growth.

In simple starvation, inadequate nutrients reaching the cells generally result in a pattern of slow weight loss, with fat tissue constituting the primary source of lost weight. A compensatory decrease in resting energy expenditure (REE) occurs with a decrease in serum triglyceride concentration. However, in wasted HIV-infected patients, a more complex pattern of wasting is observed, characterized by poor lean tissue retention, hypermetabolism, and hypertriglyceridemia (ADA, 2000, p. 432-433). According to Macallen (1999[b]) the lipid metabolism in HIV-infected adults is altered in such a way that *de novo* lipogenesis is markedly increased, which affects the way in which wasting occurs and may well contribute to the preferential lean tissue depletion. Data on the lipid metabolism in HIV-infected children are not available.

Total energy expenditure (TEE) is the sum of energy needed to maintain a child at rest, the energy needed for activities of daily living, and the thermic effect of eating. Although numerous studies have been performed on adult HIV populations, there are limited studies in paediatric HIV population which delineate resting or total energy expenditure (Miller, 1996).

Resting energy expenditure (REE) is elevated in asymptomatic HIV-infected persons (Fenton and Silverman, 2004, p. 1045; Miller *et al.*, 2001) and relates to viral load (Fenton and Silverman, 2004, p. 1045). The metabolic effects of HIV infection are induced by cytokines (Enwonwu, 1992) and lead to excessive energy expenditure (Kotler *et al.*, 1990).

In adults with HIV an active opportunistic infection appears to cause significant increases in resting metabolic rates (Miller, 1996) and protein needs; however, these HIV-induced metabolic changes and host-responses are poorly understood (Fenton and Silverman, 2004, p. 1044). According to Miller (1996) high energy expenditure may be an indication of secondary infection in HIV-infected children and may be a causative factor for accelerated weight loss. The increased metabolic rates, coupled with diminished energy intake, could be responsible for the significant weight loss in HIV-infected children.

Piozot-Martin *et al.* (1994) investigated the potential role of diet-induced thermogenesis in the aetiology of weight loss during HIV infection. Energy expenditure after food intake was more elevated in HIV-infected patients than in non-HIV controls, especially in patients with detectable clinical change in their nutritional status. The researchers came to the conclusion that both kinetics and quantitative aspects of diet-induced thermogenesis are modified by HIV infection, and the different variations are dependent on the extent of body loss.

Limited studies on total energy expenditure have been conducted in HIV-infected patients. A study by Macallen *et al.* (1999[b]) found that total energy expenditure was reduced during episodes of weight loss, and reduced energy intake was a more important determinant of weight loss in adult patients. Similar data on children are not yet available.

**2.3.2.4 Cytokines**

Altered metabolic rates, speculated to exist in children with HIV-infection, may be due, in part, to chemical messengers such as cytokines (Kotler *et al.*, 1990). The cachectin hypothesis was first postulated in 1987 by Beutler and Cerami (cited in Miller, 1996) who noticed that patients with chronic infections have significant weight loss, and that this is associated with hypertriglyceridemia and reduced clearance of triglycerides by lipoproteins. They postulated that a decrease in triglyceride clearance could lead to a decrease in fat storage and wasting of lean body mass as a result of the inability to utilize fats as a reserve source for energy. This factor was subsequently isolated and named

cachectin, later discovered to be the cytokine, TNF. Other cytokines, such as IL-1 and interferons, have similar effects (cited in Miller, 1996).

Subsequently, specific cytokines, such as TNF, IL-1 and IL-6 have been associated with infectious, inflammatory and wasting disorders. These cytokines appear to be among the body's key mediators of acute response to inflammation and infection, leading to the shunting of protein and energy sources away from the lean body compartments (Miller, 1996).

In patients with HIV, cytokines can cause ineffective use of energy substrates. Hellerstein *et al.* (1993) studied hepatic lipogenesis in HIV-infected adult patients and correlated it with peripheral cytokine levels. Both symptomatic HIV-infected patients and HIV-infected patients with weight loss had elevated hepatic lipogenesis, compared with non-infected controls. Hepatic lipogenesis correlated with interferon-alpha levels in the patients with HIV infection. In addition to increasing the hepatic synthesis of fatty acids, TNF also mobilizes free fatty acids by stimulating peripheral lipolysis. The net result is futile cycling, in which fatty acids are shuttled from adipose to tissue and back to adipose tissue, utilising energy ineffectively. The process results in increased metabolic rates, with greater caloric needs to maintain nutrition (Miller, 1996).

Cytokines may also increase HIV replication. Both TNF-alpha and TNF-beta induce HIV mRNA and reverse transcriptase by inducing transcription factors that bind to specific sites on the HIV genome (Miller, 1996). Deletion of these specific sites results in a failure of induction by TNF. This supports the hypothesis that TNF effectively increases viral replication. Thus, a positive feedback loop is established in which HIV within the macrophages induces TNF. The secreted TNF then may enhance viral replication. Thus, the cycle is completed, which effectively increases viral and TNF synthesis (Miller, 1996).

## 2.4 IMPACT OF NUTRITIONAL INTERVENTION ON HIV-INFECTED CHILDREN

HIV-infected children have special nutrition and health concerns that merit attention. Children's immature immune systems, coupled with the immune suppression effects of HIV, place younger children at a very high risk for complications such as infection and nutritional problems (Fields-Gardner and Ayoob, 2000). PEM are among the most frequent complications seen in HIV-infected children (Fenton and Silverman, 2004, p. 1051), even in the presence of HAART (Cant *et al.*, 2003, p. 1039; Grinspoon and Mulligan, 2003). For many HIV-infected children with HIV infection, death seems to be determined more by the individual's nutritional status than by any particular opportunistic infection (Sun and Sangweni, 1997). The treatment and prevention of malnutrition is essential in maintaining positive health outcomes. Strategies for nutritional care should specifically address risk factors such as decreased nutrient intake (Fields-Gardner and Ayoob, 2000).

As reduced oral intake is the most prominent factor leading to PEM in HIV-infected children, the impact of AMS on actual supplement consumption and energy intake from the AMS warrants further investigation.

In the discussion to follow the ideal characteristics of food mixtures used in nutritional rehabilitation are listed, furnishing the reader with basic knowledge on food mixtures used in rehabilitation. The impact of dietary bulk, especially on oral intake, is then discussed. Finally, the role of amylase and the benefits of added amylase are considered, and special mention is made to the impact on AMS on oral and energy intake.

### 2.4.1 Ideal characteristics of food mixtures used in nutritional rehabilitation

Low viscosity is an ideal characteristic for a food mixture to be used in rehabilitation. The ideal characteristics of food mixtures for use in community-based nutritional rehabilitation of older infants and young children; include (Brown, 1991):

- adequate energy density (≥336kJ/100g);

- adequate protein digestibility and amino acid composition;

- low osmolality (< 350mOsm/l);

- low viscosity (liquid or semi-liquid consistence – depending on age).


## 2.4.2 The impact of dietary bulk on intake


Dietary bulk covers both the volume and viscosity of food (Den Besten *et al.*, 1998). Dietary bulk, either in the form of high volume (low energy) or high viscosity (high calorie) remains a major difficulty in the nutritional management of growing children (Den Besten *et al.*, 1998; Gopaldas and John, 1992; Mitra *et al.*, 1995; Moursi *et al.*, 2003; Rahman *et al.*, 1994; Rahman *et al.*, 1995; Rahman *et al.*, 1997). Dietary bulk and high viscosity reduce oral intake (Rahman *et al.*, 1995). Viscosity can limit a child's oral intake when the consistency of the particular foodstuff exceeds a child's particular threshold to readily chew and swallow the foodstuff (Bennett *et al.*, 1999).

In developing countries one of the main staple foods are cereal-based porridges, typically made from maize, rice or sorghum (UNICEF, 1998). The starch-based staples have a low energy density (Den Besten *et al.*, 1998; Michaelsen and Friis, 1998; Mitra *et al.*, 1995) and high viscosity (Bennett *et al.*, 1999). When a cereal-based porridge is made energy-dense, the porridge is thick, sticky and has a high viscosity. Young children cannot readily chew and swallow food with high viscosity, even when they are not sick. Thus, the child's oral intake is decreased by high viscosity. On the other hand, when the staples are diluted with water to make them suitable for children to eat, the children cannot eat sufficient amounts of the high-volume, diluted staples to meet their energy requirements (Den Besten *et al.*, 1998; Mitra *et al.*, 1995; Rahman *et al.*, 1994).

The high energy requirement of young children, together with their limited stomach capacity, makes it very difficult to reach their total energy requirements with ordinary staple foods. However, the negative impact of dietary bulk on oral intake can be

overcome by liquefying an energy-dense, thick porridge with the enzyme, amylase (Gopaldas and John, 1992; Rahman *et al.*, 1994; Rahman *et al.*, 1997).

### 2.4.3   The role of amylase

Amylase is an enzyme that breaks starch-chains (amylose and amilopectin) down into smaller sugar fragments such as maltose, maltodextrins and glucose (Donnen *et al.*, 1996; Rahman *et al.* 1994). Maltose and dextrins have a low water-holding capacity and do not gelatinize on cooking, resulting in reduced swelling and a significant reduction in viscosity. Thus, a food with a low viscosity suitable for children can contain a much greater concentration of flour, and the nutrient density is more than doubled (Darling *et al.*, 1995; Michaelsen and Friis, 1998).

Simple traditional household technologies, including germination, have been used to process cereals and to improve their nutritional quality (Gopaldas and John, 1992; Michaelsen and Friis, 1998) and viscosity (Donnen *et al.*, 1996). When cereals are soaked, a process of germination, or sprouting, is started. After germination the cereal is dried and milled to flour, producing amylase-modified flour (AMF) (Delgado and Saldivar, 2000; Michaelsen and Friis, 1998; Rahman *et al.*,1994).

Germination has a profound effect on the nutritional quality of the cereal (Delgado and Saldivar, 2000; Michaelsen and Friis, 1998). Germination increases the content of some vitamins, such as vitamin C. Germination increases the amylase content of the cereal or milled flour (Delgado and Saldivar, 2000; Michaelsen and Friis, 1998).

Germination also activates native phytases and substantially degrades the phytic acid content in the cereal (Gibson, 2000; Hurell, 2003). The protein bio-availability is increased and the amount of phytic acid is decreased (Michaelsen and Friis, 1998); thereby enhancing the bio-availability of iron and zinc, copper and manganese in cereals (Gibson, 2000).

A cheap way to prepare energy-dense, low viscosity porridge is to add a small amount of AMF from germinated cereal to a thick porridge (Den Besten *et al.*, 1998; Rahman *et al.*, 1994), creating amylase-modified supplements (AMS). Small amounts of AMF can liquefy thick porridges (Weaver, 1994) and increase their nutrient density (Donnen *et al.*, 1996). AMS makes it possible to use thin, energy-dense porridges for nutritional rehabilitation for children, particularly at home (Rahman *et al.*, 1994).

### 2.4.4    The benefits of amylase on children

The modification of the dietary characteristics of bulky cereals (with the addition of amylase) has been used as a way to improve children's dietary intake and several studies have been carried out to assess the effects of modifying the viscosity (Moursi *et al.*, 2003; Rahman *et al.*, 1995); however, the impact of AMS on actual consumption and energy intake of HIV-infected children is unknown. The benefits of amylase-modified supplements on the supplement consumption, energy intake and growth in children are discussed.

Viscosity limits oral intake when the consistency of the diet exceeds some particular threshold above which children can no longer readily chew and swallow the diet (Bennett *et al.*, 1999). Decreasing the viscosity of porridges (with the addition of amylase-modified flour), allows children to chew and swallow the diet more readily, thereby increasing the oral intake and possibly the energy intake.

The feeding of AMS (high-energy dense, low viscosity porridge) to hospitalized toddlers resulted in a significant increase in the energy and protein intake (Donnen *et al.*, 1996). In India, infants and toddlers consumed significantly more AMS containing amylase than children who consumed the same supplements without the amylase. These results were confirmed in a trail in which children that were fed AMS, ate significantly more of the supplements than children who were given the supplements without amylase (John and Gopaldas, 1988). In Tanzania groundnut supplements were prepared in different concentrations and tested on 40 pre-school children. Children consumed significantly less

of the thick supplements than the thin AMS liquefied with AMF (Mosha and Svanberg, 1990).

Dysphagia and odynophagia are common amongst HIV-infected children, and limit oral intake. It can be assumed that by decreasing the viscosity of porridges (with the addition of amylase), the HIV-infected children could more easily chew and swallow AMS in comparison to thick porridges; and thereby possibly increase the AMS consumption and energy intake.

Bennett *et al.* (1999) found that amylase liquefaction of high-energy-density, high-viscosity diets increased the total daily energy intakes by young Peruvian children recovering from malnutrition or infection. In some cases, the amylase shortened the duration of feeding times. Rahman *et al.* (1994) evaluated the role of an AMS on the energy intake of severely malnourished children and infants. The study suggested that the use of AMS increased the energy intake of the children. Rahman *et al.* (1995) found that AMF-treated porridge increased the energy intake in infants and young children during acute shigellosis. Rahman *et al.* (1997) found that AMS were well absorbed in children with acute diarrhoea, and could have a positive impact in the prevention of weight loss during acute illness.

Dysphagia and odynophagia are common amongst HIV-infected children, and limit oral intake. It can be assumed that by decreasing the viscosity of porridges (with the addition of amylase), the HIV-infected children could more easily chew and swallow AMS in comparison to thick porridges; and thereby possibly increase the AMS consumption and energy intake.

John and Gopaldas (1993) compared the impact of a high-energy, low bulk diet and a high energy, high bulk diet on the growth of children. They concluded that the children who received the AMS, showed greater improvements in their growth. The researchers concluded that the much higher intake of the AMS in the experimental group was responsible for the substantial better growth.

## 2.5    SUMMARY

HIV has numerous manifestations, including malnutrition. The immune system, malnutrition and HIV are locked into a complex three-way relationship. HIV gives rise to malnutrition which, in turn, leads to a variety of immune system dysfunctions; and ineffective immunity, with its accompanying impairment of body-defence mechanisms, allows HIV to flourish. These closely linked events can initiate a vicious cycle that leads inexorably to death.

The most common foods used to feed children in under-developed countries are starchy gruels produced from traditionally refined grains such as wheat, maize and sorghum. The main problems associated with porridge prepared from cereal flour are the high viscosity and low energy and protein content.

Viscosity limits oral intake when the consistency of the diet exceeds some particular threshold, above which children can no longer readily chew and swallow the diet. Dysphagia and odynophagia are common amongst HIV-infected children and may limit the oral intake.

The modification of bulky gruels has been used as a way to improve children's dietary intake. Although several studies proved that the addition of amylase to porridges can improve oral and energy intake in toddlers, the impact of amylase on the actual AMS intake and energy intake from AMS in HIV-infected children is unknown.

# CHAPTER 3
# METHODOLOGY

## 3.1    INTRODUCTION

As stated in Chapter 1, the aim of the study is to determine the actual supplement consumption and energy intake from the supplement of institutionalized HIV-infected children.

The ethical considerations, study design, study population, stratification, measurements, intervention, pilot study, training of the caregivers, procedures of data collection, conceptual framework, statistical analysis of the data, and the limitations of the study are discussed in this chapter.

## 3.2    ETHICAL CONSIDERATIONS

Ethical approval by the Ethics Committee of the Faculty of Health Sciences was obtained (ETOVS no. 190/00C). Approval was obtained from Head Administrator (Mrs Snyman) of Lebone House to conduct the study at Lebone House, Bloemfontein (Appendix A).

Informed consent was obtained from the participants' legal guardians (Appendix B). The guardians were informed about the study and what the study entailed before the guardians were asked to sign the consent forms. The consent forms were available in Afrikaans, English and Sesotho.

## 3.3    STUDY DESIGN

The study design was a double-blinded, randomized clinical, controlled, prospective trial. The participants in the sample were stratified according to age, CD4+ counts and weight-for-age, and placed into a red and a blue group. The stratification of the groups and the

placement (discussed in 3.4) of the participants in the red (R) and blue (B) groups were done by the Department of Biostatistics, University of the Free State (UFS).

The participants in the B- and R-groups were to receive an enriched maize supplement, packaged in blue and red plastic bags, respectively. The blue (B) supplements and red (R) supplements were served as breakfast replacements to the B- and R-groups respectively. The packaging of the supplements was arranged by the first researcher; however, only the manufacturer of the maize supplements knew what colour coding was used for the experimental and control supplement respectively. Neither the researcher nor the participants were aware which colour supplement was the experimental one and which one the control supplement. The colour coding of the supplements was disclosed only after the data collection and statistical analysis had been completed.

## 3.4    STUDY POPULATION

The study population was defined as 16 HIV-infected children, resident in Lebone House, Bloemfontein at the time of the study.

### 3.4.1   Procedure and sample

Convenience sampling was used for this study. The initial study population included all 19 HIV-infected children, resident in Lebone House, at the time of the study. However, three participants were excluded from the initial sample because of long periods of absenteeism. Only 16 HIV-infected children completed the study; thus, only the data of 16 children are included in this script. The age range of the 16 HIV-infected children was between 28 months (2 years and 4 months) and 122 months (10 years and 2 months).

Only institutionalized HIV-infected children were included in the study in order to rule out food insecurity, and to minimize other variables that could influence research outcomes.

### 3.4.2   Exclusion criteria

The following were used as exclusion criteria:

- HIV-negative children;
- children with known allergies for soy, or children with an allergic reaction to soy;
- children with foetal alcohol syndrome; and
- children younger than 18 months.

The children were not denied medical treatment for HIV/AIDS during the course of the study. No children passed away during the course of the study.

### 3.5   STRATIFICATION

The stratification of the participants was done by Department of Biostatistics, UFS. The children were stratified according to their age, weight-for-age and CD4 cell counts. Information was collected during the baseline data collection (Figure 3.1).

The participants were initially divided into two age groups: children older than five years, and children five years and younger. This age group was specifically chosen in order to classify the severity of immunosuppression according to the Centres for Disease Control and Prevention (CDC) classification system (CDC, 1994). The CDC classification (Table 3.1) was used to interpret the level of immunosuppression, according to CD4 cell counts.

The children's weight-for-age scores were used for the stratification of the children. A weight-for-age Z-score of less than minus two standard deviation (< -2 SD) of the median from the National Centre for Health Statistics (NCHS) or more than -2 SD was used to differentiate between underweight and normal weight children.

The stratification allowed the Department of Biostatistics to randomize children with different characteristics (with regard to CD4 cell count, age and weight for age status)

equally between the R- and the B-group. The stratification ensured that the R- and B-groups included participants with similar characteristics.

**Table 3.1**    Immunologic categories based on age-specific CD4+ cell counts and percentages of total lymphocytes (Centres for Disease Control and Prevention [CDC], 1994)

| Immunologic category | Age of child | | |
|---|---|---|---|
| | **< 12 months** | **1-5 Years** | **6-12 Years** |
| | **Cells/mm³   (%)** | **Cells/mm³   (%)** | **Cells/mm³   (%)** |
| No evidence of suppression | ≥1,500   (≥25) | ≥1,000   (≥25) | ≥500   (≥25) |
| Evidence of moderate suppression | 750 – 1,499   (15-24) | 500 – 999   (15-24) | 200 – 499   (15-24) |
| Severe suppression | < 750   (<15) | <500   (<15) | <200   (<15) |

## 3.6    MEASUREMENTS

The variables measured included actual supplement consumption and energy intake from the supplement.

### 3.6.1   Actual supplement consumption

The actual supplement consumption is expressed as the mean amount of supplement consumed, the mean percentage of the served supplement consumed, and the percentage of days the participants consumed the entire supplement.

The mean amount of supplement consumed (in grams) refers to the mean amount of supplement consumed for each participant over all the times the supplement was served. The amount of supplement consumed is summarized per group by minima, medians, and maxima.

The mean percentage of the served supplement consumed refers to the mean percentage of supplement consumed by each participant, calculated over all the times the supplement was served. The mean percentage is summarized per group by minima, medians and maxima.

The percentage of days the participants consumed the entire supplement refers to the number of days that a participant consumed the full portion of the supplement served; expressed as a percentage of the total amount of times the supplement was served to that participant.

### 3.6.2 Energy intake from supplement

The energy intake from the supplement refers to the mean energy intake and the percentage of the energy consumed from the portion served.

The mean energy intake refers to the mean energy consumed from the total supplement served, expressed in kilojoules (kJ) and calculated over all the times the supplement was served. The mean energy intake is summarized per group by minima, medians and maxima.

The mean percentage of energy consumed from the portion supplement served refers to the mean percentage of energy consumed, calculated over all the times the supplement was served. The mean percentage of energy consumed was summarized per group by minima, medians and maxima.

### 3.6.3 Difference in supplement consumption and energy intake from the supplement between the R- and B-groups

The difference in supplement consumption between the B- and R-groups refers to the differences in the actual supplement consumption and difference in energy intake.

The differences between the B- and R-groups refer to the differences in the mean amount of supplement consumed; mean percentage of the served supplement that was consumed and the percentage of days the participants consumed the entire supplement.

The differences in the energy intake between the B- and R-groups refer to the difference in terms of the mean energy intake and the mean percentage of energy consumed from the portion served.

## 3.7 MEASURING TECHNIQUES

Different techniques to determine the actual supplement consumption and energy intake from the supplements were used.

### 3.7.1 Actual supplement consumption

A reliable and calibrated electronic scale with digital reading and an accuracy of one gram (Avery Berkel Scale, Model F100) was used for the weighing of the supplements. The valid standard weighing technique was used for the measuring of actual supplement consumption. The researcher weighed the amount of supplement served and the amount of supplement left over. The actual supplement consumption was determined by subtracting the amount of left-over supplement from the amount of supplement served. Only the researcher weighed the supplements served and the leftovers, ensuring uniformity.

### 3.7.2 Energy intake from supplement

The energy densities of both supplements were known (Table 3.2), as well as the actual portion supplement consumed (portion sizes are indicated in Table 3.3). The energy intake from the supplements was to be calculated by the Department of Biostatistics, UFS.

## 3.8 INTERVENTION

In this section the development and nutritional content of the supplement and the quantity of supplement served during the intervention are described.

### 3.8.1 The development and characteristics of the supplement

As reported in the NFCS, ninety-three percent of children in the age groups one to nine years consumed maize (MacIntyre and Labaradrios, 2000, p. 321). Maize-porridge is an affordable and culturally acceptable meal for nutritional rehabilitation.

The energy density of maize-based porridge, however, is low (Michaelsen and Friis, 1998), due to the water-binding and gelatinization properties of starch (Darling *et al.*, 1995). The high viscosity of cereal-based porridges can reduce and limit oral intake – especially in young children (Rahman *et al.*, 1995). Cereal-based porridges have a low protein content and low bioavailability of various nutrients, such as vitamin A, iron and zinc. The absorption of non-haeme iron and zinc is impaired by the high content of phytate (Michaelsen and Friis, 1998). The protein content of cereal-based porridges can be improved with the addition of legumes such as soybean. However, legumes are also rich in phytic acid, which is an additional potent inhibitor of mineral and trace element absorption, especially iron and zinc (Hurrell, 2003).

As indicated in the introduction (see 1.3), the low energy-density and low bioavailability of cereal-based porridges can be overcome with the addition of amylase. Amylase liquefies a thick energy-dense porridge to thin (low viscosity) porridge. The low viscosity enables children to more readily chew and swallow the porridge, thereby increasing the energy intake in children. Amylase-modified flour (AMF) contains activated native phytates which degrades the high phytic acid content in the cereals, thereby increasing the bioavailability of minerals (Gibson, 2000).

The first researcher (see 1.1) assisted Diva Nutritional Products with the development of a new food product, which can be described as an affordable and culturally acceptable amylase-modified, enriched maize supplement. It had the benefits of optimal viscosity (for increased intake) and optimal bioavailability (for optimal absorption). The new food product was used as the experimental product in the study. The nutritional content of the experimental and control supplement (pre-cooked, dry) is shown in Table 3.2.

**Table 3.2**      Nutritional content of the experimental and control supplements per 100g serving (pre-cooked, dry)

| Nutrient | Experimental supplement | Control supplement |
|---|---|---|
| Protein (g) | 14.0 | 14.0 |
| Total carbohydrate (g) | 61.2 | 61.2 |
| Fat (g) | 14.8 | 14.8 |
| Energy (kJ) | 1765 | 1765 |
| Isoflavones (mg) | 94 | 94 |
| Vitamin A (mcg RE) | 600 | 600 |
| Beta carotene (mg) | 0.6 | 0 |
| Vitamin D (mcg*) | 15 | 15 |
| Vitamin E (mg) | 6 | 6 |
| Vitamin C (mg) | 150 | 67.5 |
| Thiamine (mg) | 1.2 | 1.2 |
| Riboflavin (mg) | 1.1 | 1.1 |
| Niacin (mg) | 11 | 11 |
| Vitamin B6 (mg) | 1.6 | 1.6 |
| Folic acid (mcg) | 200 | 200 |
| Vitamin B12 (mcg) | 2.5 | 2.5 |
| Biotin (mcg) | 50 | 50 |
| Pantothenic acid (mg) | 4 | 4 |
| Vitamin K (mcg) | 30 | 30 |
| Sulphur (mg) | 34 | 0 |
| Beta sitosterols (g) | 30 | 30 |
| Calcium (mg) | 825 | 825 |
| Phosphorus (mg) | 660 | 660 |
| Potassium (mg) | 550 | 550 |

| Nutrient | Experimental supplement | Control supplement |
|---|---|---|
| Iron (mg) | 12.2 | 12.2 |
| Magnesium (mg) | 250 | 250 |
| Zinc (mg) | 15 | 15 |
| Iodine (mcg) | 90 | 90 |
| Manganese (mg) | 1.8 | 1.8 |
| Copper (mg) | 1.8 | 1.8 |
| Sodium (mg) | 320 | 320 |
| Molybdenum (mcg) | 25 | 25 |
| Chromium (mcg) | 25 | 25 |
| Selenium (mcg) | 40 | 40 |
| Chloride (mg) | 500 | 500 |
| N-acetyl cystein (mg) | 120 | 0 |
| Amylase | + | - |

\* mcg = µg

### 3.8.2 Quantities served during the intervention

Table 3.3 shows the adjustments in the consistency and amounts of R- and B-supplement served during the study. The adjustments in consistency were made to address the caregivers' complaints about the consistency of the supplements (see 3.14.3).

Both supplements were prepared by adding boiled water to the dry supplements and the thorough mixing of the dry supplement with the boiled water. No cooking was required, as both the supplements were pre-cooked.

The portion sizes started with 450 grams wet supplement. During the third week of the intervention, the amount of water was kept the same, but the amount of dry supplement was decreased (from 150 grams to 110 grams). In week 4 (15 September) the portion sizes for the B- and R-supplements were standardized at 521 grams wet supplement. This portion size was maintained for the rest of the study, that is until week 16.

**Table 3.3**    The portions sizes served to the study participants

| Date and week no. | Total amount of wet supplement (g) | Weight of the dry supplement (g) | Amount of water (ml) |
|---|---|---|---|
| Week 1 | 450 | 150 | 300 |
| Week 2 | 450 | 150 | 300 |
| Week 3 | 355 | 110 | 300 |
| Week 4 , day 1 | 355 | 110 | 300 |
| Week 4, day 2 until week 16 | 521 | 150 | 400 |

## 3.9    PILOT STUDY

A pilot study, which was not included in the main study, was performed at Oraratile Care Centre prior to the main study. Oraratile Care Centre was chosen because of the similarities between Oraratile Centre and Lebone House. Oraratile Care Centre also specializes in the care for HIV-infected and HIV-affected children, the children were also institutionalized, and the children's age ranges were similar.

The goal of the pilot study was to test the acceptability of the B- and R-supplements, the measuring techniques, and the documentation of the data. The pilot study was conducted over four days on all 11 children in the centre, ages ranging between three and eleven years of age. The pilot study had a double-blind, cross-over design.

The children were randomly divided into an R- and a B-group. For the first two days of the pilot study, the children each received a 450 gram portion (150 grams dry weight and 300ml water) of the R- or B-supplements, respectively. After two days, the study design was crossed over; and the children in the R-group received the B-supplement, while children in the B-group received the R-supplement. The amounts of supplements served, and the amounts of left over supplements were measured with an electronic scale and documented on the actual supplement consumption coding form (Word-document). The coding forms were slightly altered to improve the efficacy of documenting the data (Appendix C).

## 3.10    TRAINING OF CAREGIVERS

The caregivers at Lebone House received training before the main study was started. A training manual was compiled (Appendix D) to address the basic aspects of the study.

The training consisted of two one-hour sessions. The training included a short course on basic nutrition, the importance of general and food hygiene, the mixing of the supplements, measuring and weighing of the supplements, and the importance of children receiving the correct supplements. After the training was completed, the caregivers were fully informed about what the project entailed, and how to assist the researcher. However, only the researcher was involved in the weighing of the supplements served and the supplements left over.

In this study, the caregivers assisted the researcher in the marking of porridge bowls (with the participant's names); the caregivers assisted the researcher in mixing the supplements and serving the supplements. The caregivers ensured that the participants each received the correct porridge bowls (according to the names marked on the bowls). After the participants had consumed the supplements and the researcher had weighed the leftovers, the caregivers assisted the researcher to wash the dishes.

## 3.11    PROCEDURES OF DATA COLLECTION

The procedures for data collection are shown in Figure 3.1.

### 3.11.1  Initial phase

The ethical approval was obtained (see 3.2). The pilot study was performed (see 3.9) and caregivers were trained (see 3.10).

### 3.11.2 Baseline data collection

The first and second researchers were involved in the baseline data collection which took place prior to the intervention.

### 3.11.3 Measuring actual supplement intake

The participants in the R- or B-groups were supplemented with R- or B-supplements for 16 weeks. The supplements were served four times per week as a breakfast replacement. Only during the first week the supplement was served five times.

Every morning (on the days of supplementation) the researcher weighed the B- and R-groups' total mass of dry supplements. After each group's supplements were weighed, the groups' supplements were place into a red or blue mixing bowl and mixed with very hot water. The water was measured beforehand with a calibrated measuring cup. After the supplements had been mixed, the researcher dished up the R- and B-supplements into red or blue porridge bowls, respectively. The researcher weighed every participant's porridge bowl, to ensure that the precise amount of supplement was served (see Table 3.3 for the precise amounts served). The caregivers at Lebone House assisted the researcher in serving the supplements to the correct participants (according to the names on the porridge bowls). After the participants had finished eating, the researcher cleared the table, weighing all the porridge bowls with leftovers. The researcher documented the portion served (in grams), and portion leftovers (in grams) for every participant in the R- and B-groups on a standardized Word-document coding form (Appendix C).

### 3.11.4 End data collection

End data were collected by the first and second researcher and this researcher calculated the actual supplement consumption and energy intake from supplement.

## 3.12    CONCEPTUAL FRAMEWORK: A SUMMARY

The methodological procedures expounded in the above discussion are summarized in the following diagram.

```
                    ┌─────────────────────────────────────────────┐
                    │              INITIAL PHASE                    │
                    │  •  Ethical approval and informed consent*    │
                    │  •  Pilot study and training of caregivers*   │
                    └─────────────────────────────────────────────┘
                                         │
                                         ▼
        ┌───────────────────────────────────────────────────────────────────┐
        │              BASELINE DATA COLLECTION:                              │
        │  Weight, immune & health status (first researcher); anthropometrical│
        │  status (second  researcher)                                        │
        └───────────────────────────────────────────────────────────────────┘
                                         │
                                         ▼
            ┌─────────────────────────────────────────────────────────┐
            │  Stratification and randomization of participants         │
            │  according to:                                            │
            │           age, weight for age, CD4+ count                 │
            └─────────────────────────────────────────────────────────┘
                                         │
                                         ▼
    ┌──────────────┐         ╭─────────────────────╮        ┌──────────────┐
    │ 8 Red-group  │ ◄────── │    INTERVENTION     │ ──────► │ 8 Blue-group │
    └──────────────┘         ╰─────────────────────╯        └──────────────┘
                                         │
                                         ▼
        ┌───────────────────────────────────────────────────────────────────┐
        │                        MONITORING:                                  │
        │  •  Measuring daily actual supplement consumption*                  │
        │  •  Weekly anthropometric measurements (second researcher)          │
        │  •  Monthly weight, immune and health indicators (first researcher )│
        └───────────────────────────────────────────────────────────────────┘
                                         │
                                         ▼
        ┌───────────────────────────────────────────────────────────────────┐
        │                    END DATA COLLECTION                              │
        │  Calculation of actual supplement intake and energy intake from     │
        │  supplements*                                                       │
        │  Weight, immune & health status (first researcher); anthropometrical│
        │  status (second researcher)                                         │
        └───────────────────────────────────────────────────────────────────┘
```

**\* The procedures in which this researcher participated are marked with \***

**Figure 3.1**       Flow diagram of the procedures of data collection

## 3.13    STATISTICAL ANALYSIS OF THE DATA

The analysis was done by the Department of Biostatistics, UFS, using the SAS program. Numerical variables were summarized by medians, minima and maxima due to skewed distributions, and categorical variables by frequencies and percentages. For each participant the amount consumed per occasion when the supplement was served was calculated, as well as the percentage of the served supplement consumed.

Similarly the energy intake (in kJ) was calculated per occasion, and the percentage of the energy consumed from the supplement served. For each child the mean amount consumed, the mean percentage consumed, the mean energy intake and the mean percentage of energy consumed were then calculated over all the times the child was served the meal. These means were summarized per group by medians, minima and maxima. Mann-Whitney tests were performed to evaluate the statistical significance of the differences between the groups, and 95% confidence intervals (CI) calculated for differences in medians between the groups.

## 3.14    PROBLEMS ENCOUNTERED AND LIMITATIONS OF STUDY

The problems encountered and the limitations of the study are described in this section, and the possible negative influence of the limitations on the validity and reliability of the data are highlighted.

### 3.14.1  Age range of the participants

The big age range of the HIV-infected children (between 28 months and 122 months) could possibly be a limitation of the study. According to Bennett *et al.* (1999) viscosity can limit intake only when the consistency of the diet exceeds some particular threshold above which children can no longer readily chew and swallow the diet. In order to define the effect of reduction of viscosity in different situations, it is necessary to restrict a study to young children of a reasonably narrow age range (Bennett *et al.*, 1999).

The narrow age restriction, as mentioned above, is however, not applicable to this study, as this study is based only on HIV-associated reduced oral intake. HIV-associated anorexia is well described by various authors for all age groups (ADA, 2000, p. 432; Fenton and Silverman, 2004, p. 1044). The goal of the study was specifically to evaluate the impact of amylase on the supplement consumption and energy intake of HIV-infected children.  The validity of the data was not influenced by the age range.

### 3.14.2  Amounts of supplements

The amounts of supplements given to the children were not adapted for the different age groups of the participants, and could possibly be a limiting factor in this study. The portion sizes could not be increased (to more than 521 grams) for the older children, because the capacity of the colour-coded porridge bowls did not allow for bigger portions. *Ad libitum* amounts of supplements should have been implemented, but was impractical. Limited time was available to get the children ready for school in the morning, as well as the lack of personnel to assist in the serving of additional, pre-weighed *ad libitum* supplement-portions.

However, the amounts of supplements served to the participants were similar for both the B- and R-supplements. Thus, the portion sizes did not affect one of the two groups more significantly, and did not impact on the validity of the data.

### 3.14.3  Consistency of the supplements

During the second week of the intervention, the caregivers of Lebone House complained about the consistency of the supplements. There caregivers believed that the consistency was too high for the younger children to readily chew and swallow the supplements.

Unfortunately, the consistency of the supplements was adjusted during the intervention. During the second week of the intervention, the caregivers of Lebone House complained

that the consistency of both the supplements was too high. The researcher adjusted the consistency of the supplements during the third week (Table 3.3).

However, the consistency of the original portion (150 gram supplement plus 300ml water) used in the pilot study was acceptable to the participants in the pilot study. It was only after the second week of supplementation at Lebone House that the caregivers complained about the consistency of the supplements. The researcher adjusted the consistency as quickly as possible and recorded all the changes that were made to the supplements.

The objectives of the study were to determine the actual supplement consumption and energy intake from the supplements. As the changes made to the consistency of the supplements were documented, the changes in the consistency of the supplements did not influence the validity of the data, because the study objectives (supplement consumption and energy intake) could still be calculated from the data.

## 3.15    SUMMARY

The aim of the study was to determine the actual supplement consumption and energy intake from the supplements of institutionalized HIV-infected children. The study design was a double-blinded, randomized clinical, controlled prospective trail. Sixteen HIV-infected children resident in Lebone House were included in the study.

The Department of Biostatistics, UFS, stratified the participants according to the participants' baseline age, CD4 counts, and weight-for-age. After the stratification, the participants were randomized into an R- and a B-group. During the pilot study, the measuring techniques and the coding form for the documentation of the actual supplement intake was standardized.

Due to the double-blinded study design, the researcher and co-researchers made use of colour-coding to ensure validity and reliability and to avoid bias. The measurements of

the study included actual supplement consumption and energy intake from the supplements.

A valid standard weighing technique was used for measuring the actual supplement consumption, and a reliable electronic scale was used for weighing the supplements served and the leftovers. To ensure uniformity, the same researcher was responsible for the weighing of supplements. The data the researcher collected, were processed by the Department of Biostatistics, with a SAS computer program. The means were summarized per group by medians, minima and maxima. Mann-Whitney tests were performed to evaluate the statistical significance of the differences between the groups, and 95% confidence intervals calculated for differences in medians between the groups.

The big age range of the participants, the amounts of supplements served and the changes made in the consistency of the supplements were limitations of the study. However, the limitations did not affect the validity of data. Thus, the data that were obtained in this study are valid and reliable.

# CHAPTER 4
# RESULTS

## 4.1    INTRODUCTION

After the data analysis by Department of Biostatistics, the data were unblinded. The B-group was identified as the experimental group and the R-group was identified as the control group. The aim of the study was to determine the actual supplement consumption and the energy intake from the supplement from the experimental group (E-group) and the control group (C-group), and to determine the difference in supplement consumption between the E- and C-groups.

The baseline characteristics of the study population, the amount of times the researcher weighed the supplement, the actual supplement consumption and the energy intake from the supplement, are described.

## 4.2    BASELINE CHARACTERISTICS OF THE STUDY POPULATION

Sixteen HIV-infected children resident in Lebone House at the time of the study, were included in the final study. The total study population consisted of five females and 11 males (Table 4.1). The E-group consisted of three female and five male participants. The C-group consisted of two female and six male participants.

**Table 4.1**      The gender distribution of the participants

| Gender distribution between the C- and E-group | | | | | | |
|---|---|---|---|---|---|---|
| **Gender** | **E-group** | | **C-group** | | **Total** | |
| | **N** | **% (N=8)** | **N** | **% (N=8)** | **N** | **% (N=16)** |
| Female | 2 | 25.00 | 3 | 37.50 | 5 | 31.25 |
| Male | 6 | 75.00 | 5 | 62.50 | 11 | 68.75 |
| **Total** | 8 | | 8 | | 16 | |

The age range of the participants (Table 4.2) was between 28.8 months (2 years, 4 months) and 122.1 months (10 years, 2 months). The median age of the participants in the E-group was 77.8 months (age 28.8 to 117.7 months) and the median age of the participants in the C-group was 65.7 months (range 31.3 months to 122.1 months).

**Table 4.2**     The age of the participants in the study

| Age of participants in the study (months) | | |
|---|---|---|
| Age-related statistics | E-Group | C-Group |
| Maximum age | 117.4 | 122.1 |
| Median age | 77.8 | 65.7 |
| Minimum age | 28.8 | 31.3 |

The medians of the baseline weight/height status for the E- and C-groups, after randomization, are indicated in Table 4.3. Z-scores categories are used to describe the weight/ height status of children. Z-scores refer to the standard deviation (SD) of the median of the NCHS. A Z-score of minus one (-1) SD of the median corresponds to the 16th percentile on the NCHS growth charts (Stallings and Fung, 1999, p. 887); a Z-score of less than 1.65 SD is comparable to a value that is below the fifth percentile (Frisancho, 1990, p. 31) and a Z-score of -2 SD corresponds to the 2.5[th] percentile (Stallings and Fung, 1999, p. 887).

A weight-for-age (W/A) Z-score of less than -2 SD from the median of the NCHS indicates moderate underweight, whereas a height-for-age (H/A) Z-score of less than -2 SD indicates moderate stunting. A weight-for-height (W/H) Z-score of -2 SD indicates moderate wasting (Torun and Chew, 1999, p. 972).

The median of the baseline W/A Z-score for the E- and C-groups was -2.09 SD and -2.02 SD, and the median of the H/A Z-score for the E- and C-groups was -3.03 SD and -2.61 SD, respectively. The baseline W/A and H/A status of the participants in the E- and C-groups is moderately depleted (moderately underweight and stunted).

**Table 4.3**      The median of the baseline weight and height status for the groups after stratification and randomization

| Median SD of Z-scores for the baseline weight/height status of the participants | | |
|---|---|---|
| Z-score indicator | E- group | C- group |
| **W/A** | | |
| Minimum W/A | -3.39 | -4.02 |
| Median W/A | -2.09 | -2.02 |
| Maximum W/A | -1.06 | -0.82 |
| **H/A** | | |
| Minimum H/A | -3.82 | -6.43 |
| Median H/A | -3.03 | -2.61 |
| Maximum H/A | -1.58 | -0.97 |
| **W/H** | | |
| Minimum W/H | -1.54 | -2.44 |
| Median W/H | -0.41 | -0.91 |
| Maximum W/H | 1.05 | 0.48 |

The CD4+ T-lymphocyte counts of the participants were collected during baseline data collection. The CD4+ counts were used during the stratification and randomization of the participants in the E- and C-groups. After the stratification and randomization, the baseline median CD4+ count for the E- and C-group was 459 cells/mm$^3$ and 418 cells/mm$^3$, respectively.

According to the CDC immunologic category classification, young children (between 1 and 5 years) are moderately immunologic suppressed when CD4+ cell counts fall between 500 and 999 cells/mm$^3$, and are severely immunologic suppressed when CD4+ cell counts fall below 500 cells/mm$^3$. However, older children (between 6 to 12 years) are moderately immunologic suppressed when CD4+ cells counts fall between 200 to 499 cells/mm$^3$ (CDC, 1994).

Although CD4+ cell counts are not directly correlated with HIV disease progression, low CD4+ cell counts are correlated with conditions associated with immunosuppression (CDC, 1994). It is clearly evident from the median CD4+ counts of E- and C-groups that the participants in both groups were moderately to severely immunosuppressed.

## 4.3 NUMBER OF TIMES THE SUPPLEMENT WERE WEIGHED

The supplements were given four times per week, over a total period of 16 weeks, except during the first week, when supplementation were given five times. Thus, in this study, the E- and C-supplements could potentially be weighed and served 65 times per participant over a period of 16 weeks.

The number of times the researcher weighed and served the E- and C-supplements for participants is indicated in Table 4.4. The median of the number of times the E-supplement was weighed for the participants in the E-group was 61 times, and the median of the number of times the E-supplement was weighed for the participants in the E-group was 63 times. The missing values were caused by the researcher's absence (1 day), a public holiday when no supplementation was given, and the absence of different participants on various other days.

**Table 4.4** Number of times the supplements were weighed per participant in the E- and C-groups

| Number of times the researcher weighed and served the supplement | | |
|---|---|---|
| | **E-group** | **C-group** |
| Maximum number or times | 62 | 63 |
| Median number of times | 61 | 63 |
| Minimum number of times | 58 | 55 |

The maximum number of times the researcher weighed the E-supplement for a participant in the E-group was 62 out of the potential 65 times, while the minimum was 58 out of the potential 65 times.

The maximum number of times the researcher weighed the C-supplement for a participant in the C-group was 63 out of the potential 65 times, while the minimum number of times was 55 out of the potential 65 times.

The difference in the medians for the E-group were -2 times and -1 time for the C-group (calculated with the 95% confidence interval [CI]). Although there was a statistical significant difference (p=0.014) between the median of the number of times the supplements were weighed and served in the two groups, this difference was not clinically significant.

## 4.4    ACTUAL SUPPLEMENT CONSUMPTION

The actual supplement consumption is expressed as the mean amount of supplement consumed, the mean percentage of the served supplement consumed, and the percentage of days the participants consumed the entire supplement.

### 4.4.1   Mean amount of supplement consumed

For each participant, the amount of supplement consumed (in g) per sitting was calculated for each time the supplement was served. The amount of supplement consumed per sitting was calculated by subtracting the amount of left-over supplement from the portion served. The mean amount of supplement consumed for each participant was then calculated over all the times the participant was served the supplement. The means per group were summarized by medians, minima and maxima (Table 4.5).

The median of the mean amount of supplement consumed by the E-group was 489 grams per sitting. The maximum and minimum of the mean amount of supplement consumed by the E-group was 497.1 and 401.1 grams, respectively.

The median of the mean amount of supplement consumed by the C-group per sitting was 490.9 grams. The maximum and minimum (mean) amount of supplement consumed per sitting was 494.7 and 430.3 gram, respectively.

**Table 4.5**    Summary of statistics of the mean amount (in grams) of supplements consumed per group

| The mean amount of supplements consumed  (grams) per sitting | | |
|---|---|---|
| | **E-group** | **C-group** |
| Maximum of the mean amount of supplement consumed | 497.1 | 494.7 |
| Median of the mean amount of supplement consumed | 489 | 490.9 |
| Minimum of the mean amount of supplement consumed | 401.1 | 430.3 |

### 4.4.2   Mean percentage of the served supplement consumed

For each participant the percentage of the served supplement that was consumed was calculated. The mean percentage consumed per participant was then calculated over all the times the participant was served the supplement. The mean percentages were summarized per group by medians, minima and maxima and are indicated in Table 4.6.

The median for the mean percentage of supplement consumed by the E-group was 98.1 percent. The maximum and minimum for the mean percentages for the E-group were 99.9 and 78.8 percent.

The median of the mean percentage of supplement consumed by the C-group was 98.6 percent. The maximum en minimum for the mean percentages for the C-group was 99.6 percent, and 85.7 percent, respectively. The data indicate the high supplement consumption for both groups.

**Table 4.6**    Summary of statistics of the mean percentage of the supplement consumed

| The mean percentage of the amount of supplement consumed (in %) | | |
|---|---|---|
| | **E-group (%)** | **C-group (%)** |
| Maximum of mean percentage | 99.9 | 99.6 |
| Median of the mean percentage | 98.1 | 98.6 |
| Minimum of the mean percentage | 78.8 | 85.7 |

### 4.4.3   Percentage of days the participants consumed the entire supplement

The number of days that the entire served supplement was consumed was calculated for each participant. Thereafter, the percentage of days was calculated over the total number of times that the supplement was served. This was summarized per group by minimum, median and maximum values.

Table 4.7 indicates the median of the percentage of times the E-group consumed the entire served supplement (94.4 percent). The maximum and minimum of the percentages were 98.3 and 51.6 percent respectively. The median of the percentage of times that the participants in the C-group consumed the entire portions served, was 92.9 percent. The maximum and minimum percentages for the C-group were 98.4 and 66.7 percent, respectively. This data also reflect the high supplement consumption of both groups.

**Table 4.7**    The percentage of days the entire E- and C-supplement were consumed
by the participants

| Percentage of days the participants consumed the entire supplement (%) | | |
|---|---|---|
| | **E-group** | **C-group** |
| Maximum percentage of days | 98.3 | 98.4 |
| Median percentage of days | 94.4 | 92.9 |
| Minimum percentage of days | 51.6 | 66.7 |

## 4.5 ENERGY INTAKE FROM SUPPLEMENT

As the energy density (in kilojoules) of the supplements was known and the actual supplement consumption was measured (amount of supplement served minus the amount of supplement left over), the energy intake from supplements could be calculated by the Department of Biostatistics, UFS. The mean energy intake and mean percentage of energy consumed are described.

### 4.5.1 Mean energy intake

The amount of energy consumed (kJ) was calculated for each participant per sitting. For each participant the mean energy intake was then calculated over all the times the participant was served the supplement. These means were summarized per group by minima, medians and maxima.

Table 4.8 shows the median of the mean energy intake for the E-group was 2540.4 kJ. The maximum of the mean energy intake was 2586.1 kJ and the minimum of the mean energy intake was 2070.5 kJ. The median of the mean energy intake for the C- group was 2553.2 kJ and the maximum and minimum of the mean energy intake were 2576.3kJ and 2234.9kJ.

**Table 4.8**      Summary of statistics of the mean energy intake for the E- and C- groups

| The mean energy intake from the supplements consumed | | |
|---|---|---|
| | **E-group (kJ)** | **C-group (kJ)** |
| Maximum of the mean energy intake | 2586.1 | 2576.3 |
| Median of the mean energy intake | 2540.4 | 2553.2 |
| Minimum of the energy intake | 2070.5 | 2234.9 |

### 4.5.2 Mean percentage of energy consumed from the portion served

The energy intake per sitting was calculated for each participant (in kJ). Thereafter, the percentage of the energy consumed from the total served supplement was calculated. For each participant the mean percentage was then calculated over all the times the participant was served the supplement.

These means were summarized per group by medians, minima and maxima. The results are identical to the percentages of the amount of supplement consumed (see Table 4.6).

### 4.6 DIFFERENCE IN SUPPLEMENT CONSUMPTION AND ENERGY INTAKE FROM SUPPLEMENTS BETWEEN THE E- AND C- GROUPS

The differences in the supplement consumption and energy intake from the supplement between the E- and C-groups are described. Mann-Whitney tests were performed to evaluate the statistical significance of the differences between the groups, and 95% confidence intervals calculated for differences in medians between the E- and C-groups.

### 4.6.1 Actual supplement consumption

The differences between the E- and C-groups are described in terms of the mean amount of supplement consumed; mean percentage of the served supplement that was consumed and the percentage of days the participants consumed the entire supplement.

The difference between the median of the mean amount of supplement consumed for the E-group was -7.5g and for the C-group 21.3g (calculated with the 95% CI). No significant difference (p=0.83) was established between the two groups for the median of the mean amounts of supplements consumed by the participants.

The difference between the median of the mean percentage of the served supplement that was consumed, was -1.5 percent for the E-group and 4.8 percent for the C-group

(calculated with the 95% CI). No significant difference (p=0.67) was established between the E- and C-groups.

The difference in the median of the percentage of days the participants consumed the entire portion supplement served was -3.3 percent for the E-group and 11.0 percent for the C-group (calculated with the 95% CI). No significant difference (p = 0.83) was established for the difference in the median between the E- and C-group.

### 4.6.2         Energy intake from supplement

The differences in the mean energy intake and the mean percentage of energy consumed from the portion served between the E- and C-groups are described.

The mean energy intake per group was summarized by medians. The difference in the median of the mean energy intake was -37kJ for the E-group and 125.3kJ for the C-group (calculated with the 95% CI). No significant difference (p=0.67) was observed between the medians of the mean energy intake between the two groups.

The difference in the median of the mean percentage of energy consumed from the portion supplement served was -1.5 percent for the E-group and 4.8 percent for the C-group (calculated with the 95% CI). No significant difference (p=0.67) was established for the median of the percentage of energy intake between the E- and C-groups.

### 4.7   SUMMARY

Sixteen HIV-infected children were included in the study, ages ranging from 28 (2 years, 4 months) to 122 months (10 years, 2 months). The study population included five females and 11 males. The E-group consisted of three females and five males, and the B-group consisted of two females and six males.

The baseline median W/A of the participants indicated moderate underweight (E-group

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-2.09 SD; C-group -2.02 SD) and the baseline median H/A indicated moderately to severe stunting (E-group -3.03 SD; C-group -2.61 SD).

After the stratification and randomization, the median of the baseline CD4+ cell counts for the E- and C-group were 459 cells/mm$^3$ and 418 cells/mm$^3$, respectively. It is clearly evident from the median CD4+ counts of E- and C-groups that the participants in both groups were moderately to severely immunosuppressed.

The C- and E-supplements could potentially be weighed 65 times per participant throughout the course of the study. The median of the number of times the supplements was weighed for the E- and C-groups, was 61 and 63 times. This reflected a statistical significant difference ($p = 0.01$), but was not clinically significant. However, these data indicate the high quality control of the study.

No significant difference was established between actual supplement consumption between the C- and E-groups in terms of the median of the mean amount of supplement consumed ($p=0.83$); however, the median of the mean consumption of the E-group (489g) and C-group (490.9g) indicates high supplement consumption in both groups. This is also reflected in the median of the mean percentage of supplement consumed, namely 98.1 percent and 98.6 percent respectively.

The median of the percentage of times the participants consumed the entire supplement was 94.4 percent and 92.9 percent of the days for the E- and C-groups respectively. This indicates that most of the participants in both groups consumed the entire supplement on the majority of the days.

No significant difference was established between the energy intake from the supplement between the E- and C-groups in terms of the median of the mean energy intake ($p=0.67$) and percentage of energy intake ($p=0.67$); however, the median of the mean energy intake for the E-group (2540.4 kJ) and C-group (2553.2kJ) indicates that both the supplements are major contributors to the total energy intake per day.

# CHAPTER 5

# DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

## 5.1    INTRODUCTION

In this chapter the limitations of the study, the baseline characteristics of the study population, the actual supplement consumption and energy intake from the supplements, as well as the differences in supplement consumption and energy intake between the E- and C-groups are discussed. The findings are then given and recommendations are made. Finally, the conclusions are made.

## 5.2    BASELINE CHARACTERISTICS OF THE STUDY POPULATION

Protein-energy malnutrition (PEM) can be an early feature of HIV infection and is associated with a rapid decrease in CD4 cell counts and an increased rate of opportunistic infections (Guarino *et al.*, 2002). The study participants were from disadvantaged communities in the rural areas of Bloemfontein, where poor nutritional status is a common finding among pre-school children (Dannhauser *et al.*, 1996; Dannhauser *et al.*, 2000). The baseline weight and height status data of the participants, confirm the high prevalence of underweight (W/A < -2 SD), as well as stunting (H/A < -2 SD) in HIV-infected children – even when they are cared for in institutions. The median W/A (E-group -2.09 SD; C-group -2.02 SD) indicated moderate underweight, while the median H/A showed moderate to severe stunting (E-group -3.03 SD; C-group -2.61 SD). A number of studies done on HIV-infected pre-school children (between the ages of 18 to 72 months) in South Africa, also reflected high figures of malnutrition, with 28 to 50.9 percent underweight and 58 to 58.8 percent stunted (Steenkamp *et al.*, 2004;  Eley *et al.*, 2002).

Growth faltering can be an indication of a decreased immune function (Cant *et al.*, 2003, p. 1302). Wasting and stunting also contribute to an increase in morbidity and mortality in HIV-infected children (ADA, 2000, p. 429; Fenton and Silverman, 2004, p. 1044;

Johann-Liang *et al.,* 2000). The weight and height status of the participants in this study shows that they may have and increased risk for HIV disease progression and mortality.

In HIV-infected individuals CD4 cell counts are normally used as an indicator reflecting immune status. The median CD4 cell counts for the E-group (459 cells/mm$^3$) and C-group (418 cells/mm$^3$) indicated immunological suppression. The CDC (1994) classifies the severity of immunological suppression according to different age categories. Young children (between 1 and 5 years) are classified as severely suppressed when CD4 cell counts fall below 500 cells/mm$^3$, while older children (between 6 to 12 years) are classified as being moderately suppressed when CD4 cell counts fall below 500 cells/mm$^3$ (CDC, 1994). Thus, regardless of the age range of the HIV-infected children in this study (24 and 122 months), the participants' immune functions were considered to be moderately to severely suppressed.

## 5.3    ACTUAL SUPPLEMENT CONSUMPTION

Study results indicated that both the E-group and the C-group consumed large amounts of the supplement with little to no plate wasting. This was reflected by:

- the median of the mean supplement consumption of the E-group (489g) and C-group (490.9g);
- the median of the mean percentage of served supplement consumed in the E-group (98.1%) and C-group (98.6 %); and
- the high medians of the percentage of days the participants consumed the entire supplement served (E-group 94.4%; C-group 92.9%).
  These data indicate that the majority of the participants in both study groups consumed most of the supplement, most of the times that it was served.

Oral food intake is often reduced in HIV-infected children due to factors like oral ulcers, which may lead to anorexia due to odynophagia and dysphagia (Miller, 1996). Diarrhoea, which is the most common gastrointestinal manifestation in HIV infection, may also

cause loss of appetite (Fields-Gardner and Ayoob, 2000). Ways to increase the intake of energy dense foods to optimise energy intake, should therefore be explored. Amylase is an amylolytic enzyme that hydrolyzes the starch granules of thick porridge to maltodextrins and simple sugars, resulting in a significant reduction in viscosity (Darling *et al.*, 1995; Michaelsen and Friis, 1998); thus, the porridge is liquefied (Rahman *et al.*, 1994). According to Marquis *et al.* (1993) ill children suffering from diarrhoea may prefer liquids to solid food. Even when young children are not ill, they find it difficult to chew and swallow porridge with high viscosity (Den Besten *et al.*, 1998; Mitra *et al.*, 1995).

Previous studies have shown that actual supplement consumption can increase when an energy-dense supplement liquefied with amylase-modified flour (AMF) was fed to children (Gopaldas and John, 1992; Mitra *et al.,* 1995; Moursi *et al.*, 2003; Rahman *et al.*, 1995; Rahman *et al.*, 1994). The researcher, therefore presupposed that the actual supplement consumption of the participants in the E-group would increase when the E-supplement (liquefied with AMF) was fed, in comparison to the consumption in the C-group who was fed an identical supplement, but without the added amylase.

In contrast to what the literature indicated (Gopaldas and John, 1992; Mitra *et al.,* 1995; Moursi *et al.*, 2003) the supplement consumption for both groups was high (98.1% of the E-supplement and 98.6% of the C-supplement served were consumed). The outcome indicated that no significant difference for the actual supplement consumption between the E- and C-groups in terms of the mean amount of supplement consumed (p=0.83), the mean percentage of served supplement consumed (p=0.86) and percentage of days the participants consumed the entire supplement served (p=0.83), could be demonstrated.

In a similar study by Stephenson *et al.* (1994) on recovered malnourished infants (7-15 months), the liquefaction of high viscosity porridge with amylase also did not increase the porridge consumption.

In contrast with the results of the present study, Gopaldas and John (1992) found that healthy infants and children consumed significantly more *ad libitum* AMS in comparison to thick, almost solid porridge. Mahalanabis *et al.* (1993) also found that the consumption from a single *ad libitum* serving of AMS was 40 percent higher in comparison to high viscosity porridge among infants (5-12 months) with acute watery diarrhoea. In addition, Mitra *et al.* (1995) found that young children (6-23 months) suffering from diarrhoea consumed 57 percent more AMS in comparison to thick, control porridge.

The results of the above mentioned studies are not consistent, some findings showing an increase in consumption (Gopaldas and John, 1992; Mahalanabis *et al.*, 1993; Mitra *et al.*, 1995) while other studies were inconclusive (Marquis *et al.*, 1993; Stephenson *et al.,* 1994). One of the explanations for the variation in outcome could be that different viscosities of the supplements are being compared in different studies. In the study by Stephensen *et al.* (1994), the experimental porridge used, for instance, is described as an amylase-thinned, drinkable porridge, while the control supplement is described as semisolid. In the study by Gopaldas and John (1992) the experimental porridge is classified as having a "pour batter" consistency, while the control porridge is labelled as being almost solid. One variable now considered is that the high viscosities used as the control products in the studies mentioned, may make the supplements unpalatable.

The E-supplement in this study had a pour-batter consistency, while the C-supplement had a soft semi-solid consistency. It was observed by the caregivers that although there was a difference in the viscosities of the E- and the C-supplements, the C-supplement did not have an exceedingly high viscosity, still making it easy for the participants to consume. A high viscosity is defined as between 3000 and 4000mPa/s (Stephenson *et al.,* 1994). Despite the liquefaction of the E-supplement with amylase, there was no significant difference in the supplement consumption between the E- and C-groups.

The data therefore indicated a consistently high supplement consumption (94.4% and 92.9% of the days the entire E- and C-supplements were consumed by the participants),

which could be in part attributed, as demonstrated by the caregivers' opinions, to the acceptability of both the supplements' viscosities.

Another reason for the high supplement intake maybe the fact that both supplements were maize-based. Maize is the starch-based cereal most often consumed by South African children (MacIntyre and Labadarios, 2000, p. 321); thus, both the supplements were culturally acceptable.

In summary, no statistical significant difference was observed in the supplement consumption between the two groups in this population. The high supplement consumption of both groups (98.1% and 98.6% of the served supplement was consumed by the E- and C-groups, respectively) can be attributed to the acceptable consistencies of the both supplements, and both supplements being culturally acceptable.

## 5.4    ENERGY INTAKE FROM SUPPLEMENT

The energy intake of a typical breakfast served to the participants at the specific institution was determined before the baseline data collection. Portion sizes served to the different age groups were weighed on an electronic scale and the nutrient content was calculated by means of Foodfundi program. The calculated energy intake was approximately 338 kJ for children between one and three years of age and approximately 683 kJ for children seven to ten years of age.

During the intervention, the usual breakfast was replaced by the E- and C-supplements. The median energy intake from the E- and C-supplements was measured to be 2540 kJ and 2553.2 kJ respectively. This shows that the energy intake from both supplements was higher in comparison with the energy intake from a usual breakfast. Although the total daily energy intake was not determined, the researcher construed from the institution's usual menu, that both supplements made a significant contribution to the total daily energy intake.

No significant differences were observed for the medians of the mean energy intake (p=0.67) and the percentages of the energy intake (p=0.67) between the E- and C-groups. The lower feeding frequency explains why no statistical significant difference was observed in the energy intakes of the two groups.

In a study by Moursi *et al.* (2003) AMS (experimental group) and thick energy-dense supplements (control group) were served once or twice per day, according to the participants' mothers' own discretion. Although the liquefaction of the experimental supplement with amylase in this study resulted in a significant increase in energy intake from the supplement, the total daily energy intake did not differ significantly between the experimental and control group who consumed the control product with a higher viscosity.

In a number of other studies (Mitra *et al,* 1995, Rahman *et al.*, 1995, Rahman *et al.* 1994), however, the addition of amylase to sticky porridges resulted in a significant increase in total energy intake. The statistical significance of the data of these studies is explained by the higher daily feeding frequencies when the supplements were served.

Rahman *et al.* (1995) and Rahman *et al.* (1994) found that feeding AMS four times daily to young children with acute shigellosis and severe PEM increased the total energy intake. In a study by Mitra *et al.* (1995), the total energy intake was also increased by feeding amylase-liquefied supplements four times daily to children with acute diarrhoea.

According to Gopaldas and John (1992) the viscosities of the supplements also influence the energy intake from a supplement, however, Marquis *et al.* (1993) found no statistical relationship between the viscosity of weaning food (semi-solid control supplement versus a liquid experimental diet) and either the total energy intake or energy intake from the supplement.

According to Stephenson *et al.* (1994) supplementation with thick, energy-dense porridge four times daily, did not result in increased energy intakes. Even the treatment of these

thick energy-dense porridges with amylase did not increase energy intakes. Thus, insufficient data exist to relate the viscosity of supplements to the energy intake from the supplement.

In summary, the data of this study indicate that no statistical significant difference was found in the mean energy intakes of the two groups. The mean energy intake was influenced by the feeding frequency and the viscosity of the control supplement, which was, according to the caregivers, still acceptable and easy to consume.

## 5.5 FINDINGS

The data on the actual supplement consumption demonstrated that the participants consumed large amounts of supplements (E-group 489g; C-group 490g), which accounted to 98.1 percent and 98.6 percent of the E- and C-supplements served. No statistical significant differences between the E- and C-groups were observed for the actual supplement consumption. The actual supplement consumption of the groups was influenced by the viscosities of both the supplements and the cultural acceptability.

The energy intake of these HIV-infected children was increased with the addition of a single portion of enriched maize porridge to the diet, even when the supplements were served as a replacement for their usual breakfast. No difference was observed between the energy intakes of the two groups.

## 5.6 LIMITATIONS OF THE STUDY

A limitation of this study was that the total daily energy intake could not be determined for each of the participants. Although the energy intake from the supplement was determined during the study, it is not known if and how the energy intake from the supplement influenced the total daily energy intake.

If the participants' intake for lunch and dinner was not influenced by the additional energy intake from the supplement, the assumption can be made that the energy intake from the supplement can significantly increase the total daily energy intake. Unfortunately, due to logistical difficulties, the actual energy intake from lunch and supper could not be determined, and therefore the true impact of the intervention in regard to total daily energy intake could not be determined.

## 5.7    RECOMMENDATIONS

This study was the first study to be found in the discipline to evaluate the impact of AMS on the supplement consumption and energy intake of HIV-infected children. Although the study provides baseline information on the impact of AMS consumption in HIV-infected children, further research is necessary to evaluate the impact of AMS on the anthropometrical, health and immune status of HIV-infected children and to evaluate the impact of AMS on other metabolic parameters.

In future studies the problems and limitations of the present study should be addressed, including:

- the portion sizes should be age-specific and the researchers should allow for the use of additional *ad libitum* portions;
- the supplements should be given as an additional supplement between meals, and not as a meal replacement to optimize total daily energy intake;
- the feeding frequencies at which the supplements are served, should be increased to at least two to three times per day, between meals. In a community-based setting, mothers should be educated to increase the meal frequency as the mean meal frequency for children in most African countries are only two meals per day (Moursi *et al.*, 2003);
- the studies should limit the age range of the participants, in order to determine the specific age categories where AMS proves to be the most beneficial on actual consumption and energy intake; and

- the total daily energy intake of the participants should be determined in order to evaluate the impact on the supplement consumption on the total energy intake.

## 5.8    CONCLUSION

The main aim of this study was to determine the actual consumption and energy intake from supplements by HIV-infected children. As discussed in this script, the aim of the study was achieved.

The data on the actual supplement consumption demonstrated that the participants consumed large amounts of supplements (E-group 489g; C-group 490g), which accounted to 98.1 percent and 98.6 percent of the E- and C-supplements served. No statistical significant differences between the E- and C-groups were observed for the actual supplement consumption. The actual supplement consumption of the groups was influenced by the viscosities of both the supplements and the cultural acceptability.

The energy intake of these HIV-infected children was increased with approximately 2000 kJ per day with the addition of a single portion of enriched maize porridge to the diet, even when the supplements were served as a replacement for their usual breakfast. No statistical significant difference was observed between the energy intakes of the two groups.

In conclusion, this study demonstrated that reducing the viscosity of the experimental supplement with amylase did not significantly increase the supplement consumption or the energy intake in comparison to the control supplement. Both supplements were palatable and acceptable for these HIV-infected children. Both supplements also increased the total daily energy intake of the children with 2000kJ. Both supplements can therefore be used in the rehabilitation of HIV-infected children in South Africa.

Although this study is limited, it has provided important baseline information on the supplement consumption and energy intake of HIV-infected children in the rural areas of

Bloemfontein. This study has identified that this field lies fallow, and needs to be investigated further.

These researchers are still continuing the project at different institutions in the rural areas of Bloemfontein. The researchers are currently evaluating whether the addition of amylase to an enriched soy-maize blend would be more effective on the weight, immune, and health status of HIV-infected children in comparison to the cheaper control supplement without the added amylase. Future application of the research if proven to have a significant benefit may include the use of the supplement as part of existing or new feeding schemes to improve the nutritional status of HIV-infected children.

The researchers will report back the findings at the International Nutrition Congress, Durban, in September 2005.

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**APPENDIX A**      **CONSENT FORM FOR LEBONE HOUSE**

**UNIVERSITEIT VAN DIE VRYSTAAT**

**UNIVERSITY OF THE FREE STATE**

**YUNIVESITHI YA FREISTATA**

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## DEPARTMENT OF HUMAN NUTRITION

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2004/04/20

Avril Snyman

Lebone Care Centre


Dear Avril


**NUTRITION INTERVENTION PROJECT 2004: HIV POSITIVE CHILDREN**


Our conversation on 25 March 2004 has reference. The Department of Human Nutrition, at the Free State University would appreciate it if you and the board can give the necessary permission to go ahead with above-mentioned project. The aim of the project is to determine the impact of nutrition supplementation on the nutrition, health and immune status of HIV infected children.

Parents/caretakers of all children participating would be invited to sign an informed consent before admission to the study. The study will be submitted to the Ethical

Committee of the Faculty of Health Sciences at the Free State University to ensure compliance to ethical considerations and confidentiality will be ensured.

Dietitians and health personnel will monitor HIV+ children from May until November 2004 while they receive a daily fortified supplementary meal free of charge. This meal can be provided instead of the breakfast to permanent residents. Measurements will include daily evaluations on intake of the supplement; weekly weight measurements by a dietitian, monthly health screenings by a medical doctor and two blood samples (one at the start and one at the end of the study period).

The children in Lebone House would benefit from the regular screening while receiving a daily portion of high quality supplement (both products to be used already on government tender). The cost of the supplements the children will receive - free of charge - is approximately R1.70 per child per day. During the period the institution will save on the cost of breakfast as the supplement can be used as a replacement for breakfast. During the study period, no other form of nutrition intervention or supplementation (multivitamins) should take place.

We would need the following information as a matter of urgency:
- The number and ages of HIV tested children
- The number and ages of children not tested
- The current menu and portion sizes of food provided to children

Your urgent consideration to this matter would be appreciated. In case of queries, please contact Liana Steenkamp (cell: 082 8298418).

Thanking you.

Prof Andrè Dannhauser

**APPENDIX B      CONSENT FORM FOR LEGAL GUARDIANS**

**TOESTEMMING**

Hiermee verklaar ek _____wetlike voog van

_____ wat huidiglik

permanente/ deeltydse versorging by_____ontvang, dat ek my

toestemming verleen tot die deelname van hierdie kind aan die navorsingsprojek, soos

aan my verduidelik:

- Ek is versoek dat bogenoemde kind aan die projek deelneem wat uitgevoer word deur die Departement Menslike Voeding van die Vrystaatse Universiteit.

- Die doel met die projek is om die impak van verrykte mieliepap op die voedingstatus, immuunstatus en gesondheidstatus te bepaal.

- Negentien HIV+ kinders sal vir 120 dae daagliks in die week een van twee soorte voedingsupplemente in die vorm van verrykte pap ontvang. Alhoewel hulle slegs op een soort produk is, sal beide voordele vir die kinders inhou.

- Kinders sal aan die begin van die projek en aan die einde bloedtoetse moet ondergaan. Die bloedmonsters sal deur 'n mediese dokter versamel word.

- Kinders sal op weeklikse basis geweeg word en maandeliks gemeet word.

- Kinders sal twee keer per maand deur 'n dokter ondersoek word.

- Geeneen van die metings sal nadele vir die kinders inhou nie.

- Deelname aan die projek is vrywillig en mag gestaak word.

- Alle inligting is vertroulik, maar enige resultate en MIV status aan die groep sal bekend gemaak word aan ander navorsers.

- Vir die duur van die projek mag die deelnemers geen ander vorm van vitamien supplementasie gebruik nie, aangesien bykomende hoeveelhede nadele vir die kind mag inhou.

Ek is ten volle ingelig deur _____ aangaande

bogenoemde aspekte.

My toestemming word uit vrye wil verleen en ek besef ook dat ek my toestemming te

enige tyd kan herroep.

Geteken te _____ op _____ 2004.

Voog: _____Getuie: _____ .

**PERMISSION**

I, the undersigned, _____ legal guardian of

_____ Who is

currently in permanent/day care at _____ , give consent that

_____ may participate in the project explained to me:

- I have been asked that _____ may participate in this project that is carried out by the Department of Human Nutrition from the Free State University.
- The aim with the project is to determine the impact of enriched mealiemeal on the nutritional-, immune- and health status of children.
- Nineteen HIV+ children will receive one of two food supplements in the form of enriched mealiemeal for a period of 120 days. Although each child would have to use only one product for the whole period, both products would benefit the children.
- Blood samples will be collected from children at the beginning and end of the study by a medical practitioner.
- Children will be weighed every week, and measured every month.
- Children will undergo a health assessment twice per month which will be done by a medical doctor.
- None of the measurements would harm the children.
- Participation in the project is voluntary and patients may withdraw, although I was advised to let the child complete the project if possible, as it would benefit the child.
- All data would be treated confidentially, including the HIV status, but results of the group would be made available to other researchers.
- For the duration of the project no other form of vitamin supplementation may be given to the child, as it can be harmful, because they already receive adequate amounts.

I have been fully informed by _____ about the project. I hereby agree voluntarily that the child can partake in the study and realize that my permission can be withdrawn at any time.

Signed at _____ on _____ 2004.

Guardian: _____ Witness: _____ .

**TUMELLO**

Nna, _____ molebedi wa molao wa
_____ Yeo a hlokometsweng sebakeng
sa _____, ke fana ka tumello ya hore _____
a ka nka karolo projekeng eo ke e hlaloseditsweng:

- Ke kopuwe hore _____ a nke karolo projekeng ena e etswang ke lefapha la Phepo e Ntle ho tswa Univesithing ya Foreisitata.

- Maikemisetso a projeke ena ke ho sheba ditlamorao tsa phofo ya papa e matlafaditsweng, ho ho thuseng disereletsi tsa mmele le ho bophelo ba bana ka kakaretso.

- Bana ba mashome a supileng ba nang le kokwana ya HIV ba tla fuwa mofuta o le mong feela wa e mmedi ya dimatlafatsi (supplements) ka mokgwa wa papa e matlafaditsweng bakeng sa matsatsi a 120.  Le ha bana ba tla be ba sebedisa mofuta o le mong feela wa dimatlafatsi (supplements) tsena, mefuta e le mmedi e tla ba le thuso ho bana.

- Madi a tla nkuwa ho bana qalong le qetellong ya dipatlisiso tsena ke ngaka.

- Boima le botelele ba bana bo tla nkuwa beke enngwe le enngwe.

- Bana ba tla hlahlojwa ha bedi kgweding ke ngaka.

- Bana ha ba ka ke ba utlwiswa bohloko ke ho hlahlojwa le ho methwa.

- Ho nka karolo projekeng ha se qobello, mme batswadi ba ka itokolla, le ha feela ke ile ka eletswa hore ho tla thusa ngwana haholo ha nka tswella pele ho fihlela qetellong.

- Ditaba kaofela tse nkuwang ngwaneng e tla ba tsa lekunutu, empa feela di tla sebediswa ke batho ba bang ba etsang dipatlisiso.

- Nakong ena ya projeke ena ngwana ha a tlameha ho sebedisa dimatlafatsi (supplements) tse ding.  Di ka mo utlwisa bohloko hobane o tla be a se a fumana dimatlafatsi (supplements) tse mo lekaneng.

Ke tsebisitswe ka botlalo ke _____ ka projeke.

Ke dumela ka ntle le ho qobellwa hore ngwana a nke karolo dipatlisisong, mme ke utlwisisa hore tumello ya ka nka e hula nako e nngwe le e nngwe.


Signed _____ ka di _____ 2004.

Molebedi: _____ Paki: _____ .

**APPENDIX C**        **SPREADSHEET FOR DATA COLLECTION**

Date:………………………………………..Week no. …………………………..…

Group ………………........

| Name | Tuesday | | Wednesday | | Thursday | | Friday | |
|---|---|---|---|---|---|---|---|---|
| | Portion served (g) | Left over (g) | Portion served (g) | Left over (g) | Portion served (g) | Left over (g) | Portion served (g) | Left over (g) |
| | | | | | | | | |
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**APPENDIX D     TRAINING MANUAL FOR CAREGIVERS AT**

**LEBONE HOUSE**

# TRAINING MANUAL FOR THE CAREGIVERS AT LEBONE HOUSE

June 2004

# 1 THE BASIC FOOD GROUPS

Food can be divided into 3 basic food groups according to the role the food play in the body. These food groups are the body-building group; energy group and protective group.

## 1.1 Body-building Group

The food in this group contains a substance called protein. Protein is the building block of the body, and helps the body grow and heal. Body-building foods are very important for children, because the body cannot grow without enough dietary protein. Foods in the body-building group include meat, fish, chicken, eggs, cheese, milk, nuts, and legumes.
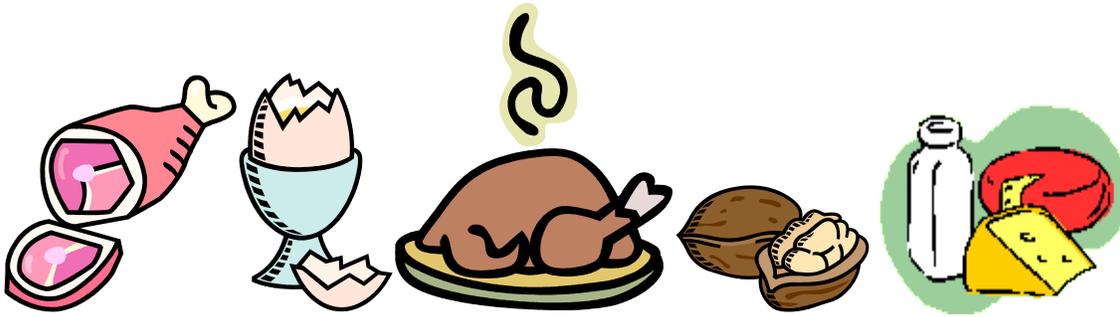


## 1.2 Energy Group

The energy group supplies the body with energy. Foods in this group act like petrol for a car. Like a car cannot work without petrol, the body cannot function without energy foods. Foods in this group include: **starch and fats.** Starchy foods include: bread, pap, bread rolls, rice, pasta, muffins, corn, wheat and potato. Fats include margarine, butter, cream, oil and mayonnaise.
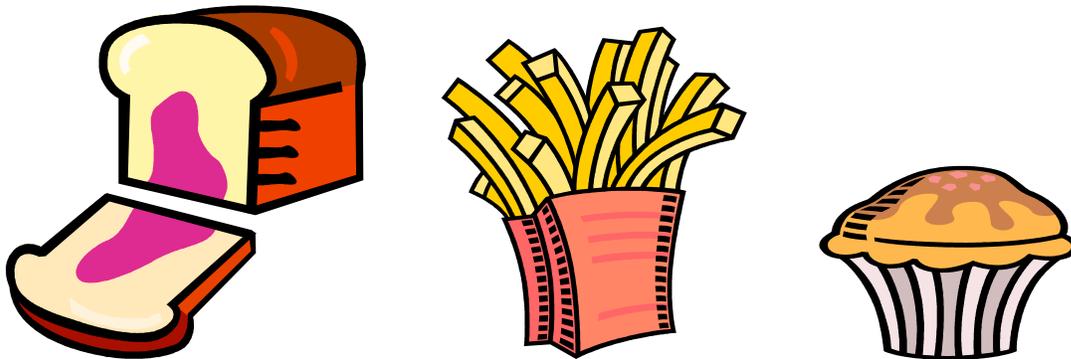
### 1.3    Protective Group

The protective group includes all types of vegetables and fruit. The protective group contains special substances (called vitamins and minerals) that help to build the body's immune system. If the body has a good immune system, the body will be less vulnerable to certain diseases.

### 2    THE IMPORTANCE OF HYGIENE

Foods contaminated with harmful germs, can cause a series of harmful diseases, such as food poisoning, infection, diarrhoea and vomiting. The body can loose a lot of fluids through diarrhoea and vomiting – causing dehydration. Dehydration can be very serious, and can be fatal in young children.

To avoid food contamination, the following basic principals should be followed:

- always wash your hands with soap and water before working with food;



- always wash your hands with soap and water after visiting the toilet;
- always wear a clean uniform/clothes and plastic gloves while you are working with food – if available;
- cover cuts/bruises on your hands with a waterproof plaster;
- always keep your hair covered with a hairnet;



- always wash fruit OR vegetables thoroughly before use;
- meat, fish OR chicken should be thoroughly cooked; never undercook meat.

- always use fresh food in the kitchen – a golden rule: *if in doubt, throw it out!*

## 3.    MIXING INSTRUCTIONS FOR SUPPLIED PORRIDGE

Two types of porridges are supplied to you. The porridge packets are colour-coded; which means that the porridge in the blue packets (referred to as the Blue Pap) should be mixed in the blue mixing bowls. The porridge in the red container (referred to as the Red Pap) is different. The porridge in the red packets should be mixed in the red mixing bowl. It is very important that the two porridges are kept apart.

**Step 1:**    Carefully weigh out the porridge. The Red Pap should be weighed apart from the Blue Pap.



**Step 2:**    After the blue and the red pap was weighed, the blue pap should be mixed with boiled water in the blue mixing bowl. The red pap should be mixed with the boiled water in the red mixing bowl. Let the porridge stand for 5 minutes.

**Step 3:**     Dish up the red porridge in the red porridge bowls. The porridge and bowls should be weighed before serving. Make sure that the porridge from the red mixing bowl is dished into the red porridge bowls. The porridge from the blue mixing bowl must be dished up into the blue porridge bowls.

**Step 4:**     Serve the porridge bowl to the correct child. The bowls are clearly marked with the child's name. It is extremely important that every child receive his/her porridge bowl.



**Step 5:**     After the children finished eating, the researcher will collect all the porridge bowls. The researcher will weigh the porridge bowls with any porridge left over in the bowls. The researcher will document the amount of left over porridge.