IMPACT OF AN ENZYME-MODIFIED ENRICHED MAIZE SUPPLEMENT ON THE GROWTH, IMMUNE AND HEALTH STATUS OF HIV+ CHILDREN

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DECLARATION

I hereby declare that the thesis submitted by me for the Philosophiae Doctor in Dietetics at the University of the Free State is my own independent work and has not previously been submitted by me to another university/faculty. I further cede copyright of this research report in favour of the University of the Free State.

Juliana Steenkamp

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AIDS	**Acquired Immune Deficiency Syndrome**
ARVs	**Antiretroviral drugs**
BMI	**Body Mass Index**
CDC	**Centres for Disease Control**
CRP	**C-reactive protein**
ELISA	**Enzyme-linked immunosorbent assays**
EN	**Enteral nutrition**
FDC	**Follicular dentritic cells**
HAART	**Highly active antiretroviral therapy**
HAZ	**Height-for-age z-score**
HIV	**Human immunodeficiency virus**
IgG	**Immunoglobulin G**
LIP	**Lymphoid interstitial pneumonitis**
MTCT	**Mother-to-child transmission**
NAC	**N-acetyl cysteine**
NAIDS	**Nutritionally acquired immune deficiency syndrome**
PCP	***Pneumocystis carinii* pneumonia**
PCR	**Polymerase chain reaction**
PEM	**Protein energy malnutrition**
REE	**Resting Energy Expenditure**
RNA	**Ribonucleic acid**
RT	**Reverse transcriptase**
RTUF	**Ready-to-use therapeutic food**
RDA	**Recommended Dietary Allowance**

SAVACG	**South African Vitamin A Consultative Group**
SD	**Standard deviation**
TEE	**Total energy expenditure**
TLC	**Total lymphocyte count**
TPN	**Total parenteral nutrition**
WAZ	**Weight-for-age z-score**
WHO	**World Health Organisation**
WHZ	**Weight-for-height z-score**

CHAPTER 1

CAN SUPPLEMENTATION WITH AN ENZYME-MODIFIED ENRICHED MAIZE PRODUCT BE OF BENEFIT TO CHILDREN LIVING WITH HIV/AIDS?

Background

For a number of years, various kinds of instant enriched maize supplements have been used as part of nutrition intervention strategies in Southern Africa. More recently the South African Department of Health, in an attempt to benefit HIV-infected adults and children in particular, has increased funding of the Nutrition Directorates in the different provinces to ensure the supply of nutrition supplements to people living with HIV/AIDS.

Instant enriched maize supplements are part of the available supplements currently included in government tender RT9. This tender is utilised, amongst others, to procure supplements for children with malnutrition or those at risk, i.e. living with HIV/AIDS. These supplements are used at primary health care level as well as selected institutions where AIDS orphans receive care and support. Some health workers are of the opinion that children in care centres do not need additional supplements as, unlike the majority of HIV-infected children in the community, they do not suffer from food insecurity and have access to regular meals of good quality. Until recently, no attempts were made to determine the impact of supplementation with instant enriched maize products on the growth, immune and health status of HIV-infected children and no information is available regarding the growth, immune and health status of children in care centres.

This study formed part of a larger study in which four researchers participated.

Researcher 1, (referred to as this researcher), determined:

- ***Baseline data with regard to growth, immune status, micronutrients/ antioxidants linked to immune status, as well as health status in HIV-infected children in care centres;***
- ***Correlations between these different indicators;***
- ***The impact of an enzyme-modified, enriched maize-based supplement (Experimental Product) on the growth, immune and health status of these children.***

Researcher 2, (fieldworker for the purpose of this study), determined the impact of the Experimental and Placebo Products on the anthropometric nutritional status of these children.

Researcher 3, (fieldworker for the purpose of this study), determined the supplement consumption and energy intake of these children receiving the Experimental and Placebo Products.

Researcher 4, (fieldworker for the purpose of this study), determined the health status of the children.

Research outcomes in respect of Researcher 1 will be described in this thesis.

1.1 Introduction

South Africa is home to about one percent of the world's population, yet 32 percent of people living with the human immunodeficiency virus (HIV) infection worldwide, reside in South Africa and 34 percent of Acquired Immune Deficiency Syndrome (AIDS) deaths occur here (UNAIDS, 2006). It was estimated that at the end of 2004, 6.29 million South Africans were living with HIV, which included 104 000 children - 90 percent as a result of mother-to-child transmission (MTCT). The HIV prevalence among pregnant women in 2005 was observed to be 30.2 percent in comparison to 22.4 percent in 1999. With the highest prevalence rate in the age groups 20 to 35 years of age, MTCT still threatens the HIV status of children and remains a public health concern (Department of Health, 2006; Allen et al., 2000). In contrast with children everywhere else in the world, children in Southern Africa have a shorter life expectancy than their grandparents (UN, 2001).

1.1.1 The impact of HIV infection on nutrition

HIV infection can cause malnutrition, which contributes to immune dysfunction, thereby increasing the risk for opportunistic infections. The HIV infection, nutritional status and immune function are thus in a close relationship, each of the factors influencing the others. The dominant effect in this relationship is the effect of the HIV infection on nutritional status. In adults, this effect manifests itself primarily as wasting, especially in the absence of anti-retroviral drugs (Macallan, 1999). In 1987, the HIV wasting syndrome was included by the Centres for Disease Control (CDC) as an AIDS defining illness. It refers to weight loss of more than ten percent from baseline, with either diarrhoea or fever for more than 30 days (CDC, 1987). The Body Mass Index (BMI) at the time of HIV diagnosis has been shown to be a strong, independent predictor of

survival in HIV-infected adults in West Africa and it has been shown that the degree of weight loss is correlated with early death (Van der Sande et al., 2004).

Similar information is not freely available for children. However, we know that the nutritional implications of HIV infection for children are often more devastating than for adults, because children have an added nutritional demand of growth and development.

Weight loss in HIV-infected individuals can typically fall into two categories. Firstly, children may experience slow and progressive weight loss or growth failure, due to anorexia, gastrointestinal disturbances, psychosocial factors, economic factors and adverse effects due to treatment. Secondly, episodes of acute weight loss may result in wasting, which is usually associated with opportunistic infections (Heller et al., 2000; Kotler, 1999; Macallan, 1999; Coodley et al., 1994). In HIV-infected children younger than 13 years, the CDC defines wasting as weight loss of more than ten percent from baseline; downward crossing of at least two percentile lines on the weight for age chart in a child one year or older; or less than the fifth percentile on the weight for height chart on two consecutive measurements at least 30 days apart, plus chronic diarrhoea or documented fever for at least 30 days, whether intermittent or constant (CDC, 1994).

Results from a number of studies (Steenkamp et al., 2004; Eley et al., 2002; Eley & Hussey, 1999) amongst HIV-infected children in South Africa, showed figures of more than 25 percent for the prevalence of underweight and 55 to 60 percent for stunting. These were much higher than the average percentages in a national nutrition survey done on children below six years of age, which indicated that ten percent of children were underweight and 23 percent stunted (SAVACG, 1996). The HIV nutritional status data in South Africa also reflects international data, which indicates that chronic

malnutrition in the form of stunting is a much bigger problem in the majority of HIV-infected children older than two years, than acute malnutrition in the form of severe wasting.

1.1.2 How does nutritional status impact on immune status and health?

Studies as summarised by Salomon et al. (2002) indicated that, despite several advances that have been made in the management of HIV infection, including antiretroviral drugs, prophylaxis, treatment of opportunistic infections and psychosocial care, malnutrition remained an important prognostic factor, even in developed countries. Wasting, in particular the loss of lean body mass, has been associated with increased mortality (Kotler et al., 1989), accelerated disease progression (Wheeler et al., 1998), and impairment of strength and functional status (Gripspoon et al., 1999).

In HIV-infected children, AIDS is associated with an impaired nutritional status, growth failure and weight loss, which may further contribute to the already compromised immune system and treatment failure. In pediatric AIDS, the nutritional status and, in particular, growth faltering seems to be of greater prognostic value than any particular opportunistic infection (Sun & Sangweni, 1997).

However, impaired nutritional status is not the only factor that influences the progression of HIV infection. According to Baum et al. (1995), the progression of HIV infection may be influenced by a number of factors including co-infection with other viruses, age and use of anti-retroviral drugs. It also seems that the markers of disease progression (amongst other $CD4^+$ cell counts) are influenced by changes in nutritional status. As with adults, loss of $CD4^+$ lymphocytes is a major predictor of HIV disease progression in

children. In a study reported by Taha et al. (1999), 100 percent of HIV-infected children with a CD4 percentage of less than 15 percent of total lymphocytes, died by 32 months. In comparison to the former, only 32 percent of infected children died in the group with a CD4 percentage between 15 and 24 percent. The number of AIDS deaths dropped further to 27 percent in the group with a CD4 percentage of more than 25 percent of total lymphocytes. It is therefore evident that preservation of $CD4^+$ cell counts should be one of the primary goals in the management of HIV-infected children.

The presence of micronutrient deficiencies can also potentially affect transmission as well as the clinical course of infection (Friis & Michaelsen, 1998). As summarised by Steenkamp and Visser (2002), international data clearly indicate a link between the intake of vitamins A, C, B1, B2, B3, B6, E as well as selenium, zinc, N-acetyl cysteine (NAC) and disease progression or mortality.

1.1.3 Gaps regarding nutrition-related research in HIV-infected children

Very little data are available regarding the impact of micronutrient supplementation on children.

Henderson et al. (1997) reported that a few plasma markers, vitamin A, selenium, zinc and protein, remained normal in the absence of growth retardation and that any deficiencies might be more a result of malnutrition than a result of the HIV infection. Fawzi et al. (1999) reported improvements in $CD4^+$ cell counts in HIV-negative children after large dose vitamin A supplementation. Similar trials have not been reported regarding HIV-infected children.

According to Semba (1998), ”further examination of micronutrient intervention for HIV infection should be an extremely high priority, given the low cost of micronutrient supplements and the high potential cost/benefit ratio.” The unfortunate truth is that five years down the line, according to a working document for the World Health Organisation (WHO), apart from vitamin A, no results from randomised trials examining the efficacy of micronutrient supplementation of children, were available (Fawzi, 2003).

1.1.4 Nutrition support of children living with HIV/AIDS in developing countries

Most HIV-positive patients need nutritional support at one stage or another. In poverty stricken areas like Africa, with a high prevalence of HIV infection and malnutrition, it seems unlikely that most individuals would be in a situation where they would have constant access to a high quality diet. Therefore, a suitable food supplement might prove an ideal carrier for macro and micronutrients.

Multiple food fortification (addition of several nutrients to the same food) would be advantageous if there is a need to address several micronutrient deficiencies simultaneously at a minimum additional cost (Mora, 1995). Multiple micronutrient deficiencies also tend to coexist in the same population (as in HIV-infected/AIDS patients) and a comprehensive approach to micronutrient fortification may be more cost-effective than alternative regimens (Sun & Sangweni, 1997). Until successful prevention of the spread of the HI-virus becomes a reality, the most pressing problem in vulnerable African communities, particularly the children, is the implementation of timely, aggressive nutrition support to help prolong the period of clinical latency of the infection and forestall development of severe complications (Enwonwu, 1992).

Although dietary counselling or provision of an optimal diet is important in the management of HIV-infected patients, this is generally not the solution in developing countries harboring the majority of the world's HIV-infected individuals. Even in the technically developed world, maintenance of optimal nutritional health is exceedingly difficult. The guidelines prepared by the US National Task Force on Nutrition in AIDS, emphasise the prevention of protein energy malnutrition (PEM) as one of the major goals in individuals testing positive for HIV infection. These guidelines are, however, not readily applicable to impoverished communities where PEM is prevalent and often precedes the onset of HIV infection (Enwonwu, 1992). A staple food (or supplement based on a staple food) can achieve a high coverage rate due to good compliance by the target group. Also, because of the difficulty young children have taking pills or capsules, supplements in powdered form are often easier to consume.

Improved nutrition may play a role in limiting mother-to-child HIV-transmission, progression of HIV to AIDS, as well as AIDS mortality. There is sufficient evidence to show that there may be great promise in using micronutrients to improve maternal and child health in developing countries (Semba & Tang, 1999). Furthermore, there is emerging evidence that pediatric HIV infection in the United States has evolved from a rapidly progressive fatal disease to a chronic infection with prolonged survival (Laufer & Scott, 2000). In view of the relationship between malnutrition and progression of HIV infection to AIDS, and associated increased costs of hospitalisation and treatment, attempts at providing affordable measures to support HIV+ children nutritionally, would make medical and economic sense.

One of the challenges in developing countries like South Africa is to identify low-cost interventions, like nutritional support by using affordable, instant enriched maize products, to reduce the burden of HIV-related disease. It is, however, of utmost importance to evaluate the impact of these products on nutritional and immune status as well as disease state and outcome. This is reiterated by Lepage et al. (1998) "... it is time to move to intervention studies in the field of pediatric HIV infection in developing countries, despite the limited observational descriptive and prognostic studies thus far. This is essential because although the burden of pediatric HIV infection may be alleviated in future, it will not disappear."

1.2 Problem statement

Supplementation issues in children with malnutrition, especially those who are HIV-infected, have been an area of discussion amongst health workers for some time. Current literature (Bateman, 2004) shows that 45 percent of HIV-infected children in South Africa live in homes where there is inadequate money for food. In contrast, HIV-infected children in care centres have access to regular balanced meals, thereby limiting the impact of food insecurity, so often found in the community. Some health workers are even of the opinion that no additional macronutrient supplementation is warranted for children in these institutions and that the hospital or standard diet should be sufficient to meet all macronutrient needs required for growth. However, no clinical trials have been done to support such beliefs and no data are available on the growth, immune and health status of children in care centres.

In addition, very little data are available on correlations between growth, immune and health status in HIV-infected children in general, especially in South Africa. Even if the

HIV-infected children in care centres would present with growth failure, the question remains whether their immune and health status are significantly influenced by the growth patterns. A few studies (Arpadi et al., 2000; Berhane et al., 1997) indicated a possible link between growth failure and an increase in viral load or poorer disease outcome. If children in care centres show similar tendencies, efforts should be made to reverse the growth failure in order to decrease the viral load.

The only anti-oxidant extensively researched in children is vitamin A. Very little data are available regarding anti-oxidant levels in HIV-infected children in Southern Africa, with no available data on levels in children in care centres. It is important to determine whether deficiencies in fact exist – despite access to a balanced diet – and whether anti-oxidant levels can be correlated with growth, immune and health parameters in these children.

Targeted food supplementation for malnourished children and underweight pregnant- and lactating women, utilising different forms of instant enriched maize products, has been in operation for the past few years in Southern Africa. As part of the government's anti-retroviral roll out plan, supplemental meals (instant enriched maize products) for HIV-positive individuals, has been implemented by the National Nutrition Directorate (Department of Health, Integrated Nutrition Programme, 2004).

In the Free State Province, the nutrition protocol (2004) for optimal management of HIV-infected children, included supplementation with enriched maize meal as well as a multi-vitamin supplements.

Although it is difficult to demonstrate the benefits of nutritional support regarding clinical outcome in HIV-infected children or adults, research has shown that nutritional status and thus nutritional support, may affect both the progression of HIV-disease as well as the survival of HIV-infected individuals (Piwoz & Preble, 2000; Macallan, 1999). Efforts should therefore be made to determine the impact of such interventions in care centres as well.

Two problems have been associated with the use of maize/soy blends as a supplement of choice, in some parts of Africa and need to be investigated:

1. It has been shown that the maize/soy blends traditionally used as part of nutritional support or supplementation regimens in Africa contain large amounts of phytic acid which is usually regarded as an anti-nutritive factor (Mihihane & Rimbach, 2002). These anti-nutritive factors may undermine absorption of micronutrients (Lönnerdal, 2002), which in the case of HIV-infected children may further compromise their nutritional and immune status.

2. Maize/soy blends can also have a high viscosity, which may decrease intake of the reconstituted products – especially if taken by patients presenting with anorexia.

Addition of amylase to a soy/maize supplement consumed by malnourished children in India has proven that energy intake can also be increased as a result of lowering the bulk and increasing the energy density of weaning foods (Gopaldas & John, 1992). Manipulation of maize/soy blends by the addition of enzymes including amylase or

processes like fermentation, may increase intake and/or bioavailability of essential nutrients and positively impact on the health status of HIV-infected children.

Despite the nutrition intervention strategies implemented by Government as described previously, no attempts were made in the past to determine the impact of these supplements on the nutritional, immune and health status in children. Results from a pilot study reported by Van der Walt et al. (2004), indicated better weight gain in supplemented HIV-infected and affected children, when using an enzyme-modified fortified maize supplement, than with the unmodified enriched maize product currently available in the Free State Province. In order to implement sound nutrition strategies and thereby impact on the nutritional and health status of HIV-infected children, information is needed with regard to the current nutritional status of clinically stable HIV-infected children.

Current literature thus shows that:

- No information is available regarding the growth, immune, micronutrient and health status of HIV-infected children in care centres;
- Very little South Africa data are available regarding possible correlations between growth, markers of immune status and health status in HIV-infected children;
- Despite supplementation regimens (which include the use of maize/soy blends) in a number of the provinces, no results from clinical trials are available evaluating the impact of supplementation with enzyme modified or unmodified maize supplements.

1.3 Aim and objectives

This study was performed with the following aims in mind:

1.3.1 To describe baseline data regarding growth, immune status, micronutrients linked to immune status and health status for HIV-infected children in care centres in Mangaung.

1.3.2 To determine correlations between growth, immune, micronutrient and health status in HIV-infected children in care centres.

1.3.3 To determine whether regular intake of an enzyme-modified maize supplement (the experimental product) by HIV-infected children in care centres, could produce significant improvements in the growth, immune and health status if compared to an unmodified maize product.

1.4 Role of this researcher

In order to achieve the objectives described above, this researcher was responsible for the following aspects during the study period:

- Initiation of the study and development of study design;
- Development of the experimental product* together with a food technologist;
- Development of the outline of the full project involving three other researchers;
- Motivation for financial support and control of budget;
- Liaison with company regarding necessary payments of expenses;

- Liaison with company regarding formulation of products for pilot study and intervention;
- Co-ordination of associated studies of two researchers;
- Liaison with Sunflower house and Lebone house regarding approval to proceed and obtain information (Appendix A);
- Development of training and procedure standards;
- Investigation of procedures for standardisation;
- Training of research assistants in methods;
- Regular communication with fieldworkers in terms of problem solution;
- Development and standardisation of questionnaires;
- Supporting role to other two researchers regarding piloting and data collection; and
- Collation and interpretation of all results and liaison with biostatistician to request specific correlations.

**** This researcher was not directly involved with any physical distribution of the experimental and placebo products, to ensure unbiased results.***

1.5 Benefits of the study

This study provided vital information on the growth, immune, micronutrient and health status of HIV-infected children in care centres and possible correlations between these variables. It also indicated whether nutrition intervention could significantly benefit the growth, immune- and health status of children in care centres. As the experimental product with the enzymes was tested against a placebo product of equally high quality (exactly the same micronutrient profile), but without the enzymes, the question was

answered whether the addition of enzymes to an enriched soy-maize blend is of significant benefit to HIV-infected children. The results will be used as part of the evaluation of current nutrition intervention strategies for people with HIV/AIDS, as implemented by the National Nutrition Directorate. The benefits of the study thus include the following:

- New available information with regard to the growth, immune, micronutrient and health status of HIV-infected children in care centres;
- If it is found that these children are malnourished and/or present with micronutrient deficiencies, recommendations will be made to health workers to implement suitable nutrition intervention strategies;
- If intervention with the enzyme-modified supplement results in improved growth and/or micronutrient status, recommendations will be made to use enzyme-modified supplements as products of choice, rather than unmodified maize supplements.

1.6 Outline of this thesis

Chapter 1 provides the motivation for the study, as well as the problem statement, aim and objectives and a short summary of the role of this researcher. An outline of the thesis is also included. A literature review in support of the study is done in Chapter 2. Chapter 3 contains the methodology of the study which includes a description of the operational definitions, choice and standardisation of methods, measuring techniques, validity and reliability, population and sampling, study procedures and statistical analysis. Motivation for the development of the experimental product is also discussed.

Chapter 4 describes the baseline data on the growth, immune, micronutrient and health status of HIV-infected children in care centres in Mangaung. Chapter 5, “Correlations between growth, immune, micronutrient status and metabolic indicators in HIV-infected children in care centres in Mangaung, South Africa”, contains data on whether any correlations could be demonstrated amongst the baseline data obtained from the sample. Chapter 6, “Impact of nutrition intervention with an enzyme-modified maize/soy blend on the growth, immune, micronutrient and health status of HIV-infected children in care centres in Mangaung, South Africa – a randomised trial”, describes the changes in growth, immune status and health status indicators as a result of the nutrition intervention.

Chapter 8 contains a general summary, conclusions and recommendations. Summaries in both English and Afrikaans are also included in the thesis.

1.7 References

Allen, D.M., Simelela, N.P. and Makubalo, L. 2000. Epidemiology of HIV/AIDS in South Africa. The South African Journal of HIV Medicine, 1: 9 – 11.

Arpadi, S.M., Cuff, P.A., Kotler, D.P., Wang, J., Bamji, M., Lange, M., Pierson, R.N. and Matthews, D.E. 2000. Growth velocity, fat-free mass and energy intake are inversely related to viral load in HIV-infected children. Journal of Nutrition, 130: 2498 – 2502.

Bateman, C. 2004. AIDS Pandemic – Still time to prepare for the orphan tide. South African Medical Journal, 94(7): 505 – 507.

Baum, M.K., Shor-Posner, G., Lu, Y., Rosner, B., Sauberlich, H.E., Fletcher, M.A., Szapocznik, J., Eisdorfer, C., Buring, J.E. and Hennekens, C.H. 1995. Micronutrients and HIV-1 disease progression. Acquired Immuno Deficiency Syndromes, 9(9):1051 – 1056.

Berhane, R., Bagenda, D., Marum, L., Aceng, E., Ndugwa, C., Bosch, R.J. and Olness, K. 1997. Growth failure as a prognostic indicator of mortality in pediatric HIV infection (Abstract). Pediatrics,100(1): E7.

Centre for Disease Control and Prevention (CDC). 1987. Revision of the CDC surveillance case definition for Acquired Immunodeficiency Syndrome, MMWR, 36 (S1): 1S – 15S.

Centre for Disease Control and Prevention (CDC). 1994. 1994 Revised Classification System for HIV Infection in Children less than 13 years of age. Official Authorized Agenda: Human Immunodeficiency Virus Infection Codes and Official Guidelines for Coding and Reporting ICD-9-CM, Morbidity and Mortality Weekly Report, 43(RR-12): 2-3.

Coodley, G.O., Loveless, M.O. and Merril, T.M. 1994. The HIV wasting syndrome: A review. Journal of Acquired Immune Deficiency Syndromes, 7(7): 681 – 692.

Department of Health. 2006. National HIV and Syphilis Antenatal sero-prevalence survey in South Africa, 2005.

Department of Health. 2004. Integrated Nutrition Programme, Issue 4.

Eley, B. and Hussey, G. 1999. Nutrition and human immunodeficiency virus infection in children, South African Medical Journal, 89: 190.

Eley, B., Sive, A., Kossew, G., Abelse, L., Cooper, M. and Hussey, G. 2002. Growth and micronutrient disturbances in stable, HIV-infected children in Cape Town. Annals of Tropical Paediatrics, 22: 19 – 23.

Enwonwu, C.O. 1992. Interface of malnutrition and HIV infection in Sub-Saharan Africa: A critical review. Nutrition Research, 12: 1041 – 1050.

Fawzi, W.W., Mbise, R.L., Hertzmark, E., Fataki, M.R., Herrera, M.G., Ndossi, G. and Spiegelman, D. 1999. A randomised trial of vitamin A supplements in relation to mortality among human immunodeficiency virus-infected children in Tanzania. Pediatric Infectious Disease Journal, 18: 127 – 133.

Fawzi, W. 2003. Micronutrients and HIV-1 Disease Progression among Adults and Children. Working paper for World Health Organisation, May 2003, Geneva.

Friis, H. and Michaelsen, K.F. 1998. Micronutrients and HIV infection: A review. European Journal of Clinical Nutrition, 52: 157 – 163.

Gopaldas, T. and John, C. 1992. Evaluation of a controlled 6 Months feeding trial on intake by infants and toddlers fed a High Energy-Low Bulk Gruel versus a High Energy-High Bulk Gruel in addition to their habitual home diet. Journal of Tropical Pediatrics, 38: 278 – 283.

Gripspoon, S., Corcoran, C. and Rosenthal, D. 1999. Quantitative assessment of cross-section al muscle area, functional status and strength in men with AIDS wasting syndrome. Journal of Clinical Endocrinology and Metabolism, 84: 201 – 206.

Heller, L., Fox, S., Hell, K.J. and Church, J.A. 2000. Development of an instrument to assess nutritional risk factors for children infected with HIV. Journal of the American Dietetic Association, 100(3): 323 - 329.

Henderson, R.A., Talusan, K., Hutton, N., Yolken, R.H. and Caballero, B. 1997. Serum and plasma markers of nutritional status in children infected with the human immunodeficiency virus. Journal of the American Dietetic Association, 97: 1377 – 1381.

Kotler, D.P., Tierny, A.R., Wang, J. and Pierson, R.N. 1989. Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS. American Journal of Clinical Nutrition, 50(3): 444 – 447.

Kotler, D.P. 1999. Nutrition and wasting in HIV infection. HIV Clinical Management, Medscape, Inc., 8: 1 – 33.

Laufer, M. and Scott, G. 2000. Medical Management of HIV Disease in Children. The Pediatric Clinics of North America, 47(1): 127 -153.

Lepage, P., Spira, R., Kalibala, S., Pillay, K., Giaquinto, C., Castebon, K., Osborne, C., Courpotin, C. and Dabis, F. 1998. For the international working group on mother-to-child transmission of HIV, Care of HIV-infected children in developing countries: Report of a workshop for clinical research. Pediatric Infectious Diseases Journal, 17(7): 581 - 586.

Lönnerdal, B. 2002. Phytic acid – tracel element (Zn, Cu, Mn) interactions. International Journal of Food Science and Technology, 37: 749 – 758.

Macallan, D.C. 1999. Nutrition and immune function in human immunodeficiency virus infection. Proceedings of the Nutrition Society, 58: 743 – 748.

Minihane, A.M. and Rimbach, G. 2002. Iron absorption and the iron binding and anti-oxidant properties of phytic acid. International Journal of Food Science and Technology, 37: 741 – 748.

Mora, J.O. 1995. Issues in food fortification, Lessons from Latin America. Nutriview, 4: 1 - 8.

Piwoz, E.G. and Preble, E.A. 2000. HIV/AIDS and nutrition: A Review of the Literature and Recommendations for nutrition care and support in Sub-Saharan Africa, Washington DC: SARA Project Bureau for Africa, Office for Sustainable Development, US Agency for International Development.

Salomon, J., De Truchis, P. and Melchior, J.C. 2002. Nutrition and HIV infection. British Journal of Nutrition, 87: S111 – S119.

South African Vitamin A Consultative Group (SAVACG). 1996. Anthropometric, vitamin A, iron and immunization coverage status in children aged 6 – 71 months in South Africa, 1994. South African Medical Journal, 86: 354 – 357.

Semba, R.D. and Tang, A.M. 1999. Micronutrients and the pathogenesis of HIV infection. British Journal of Nutrition, 81: 181 – 189.

Semba, R.D. 1998. Bridging the nutrition gap – The role of nutrition in vertical transmission of HIV. SAJPH, 88(8):1020 – 1022.

Steenkamp, L. and Visser, M. 2002. Can nutrition support prevent HIV disease progression and delay AIDS related mortality? The Specialist Forum, 2(5): 44 – 54.

Steenkamp, L., von der Marwitz, J. and Giovanelli, C. 2004. Nutritional status of HIV+ pre-school children in South Africa – Summary of unpublished research. Field Exchange, Emergency Nutrition Network. [Internet]. Available from: http://www.enonline.net/fex/22/fex22.pdf>[Accessed 12 October 2006].

Sun, W.Y. and Sangweni, B. 1997. Rationale for nutrition support of children with HIV/AIDS from socioeconomically disadvantaged families. Journal of Applied Nutrition, 49(4): 88 – 93.

Taha, T.E., Kumwenda, N.I., Broadhead, R.L., Hoover, D.R., Graham, S.M., Van der Hoven, L., Markakis, D., Liomba, G.N., Chiphangwi, J.D. and Miotti, P.G. 1999. Mortality after the first year of life among human immunodeficiency virus type 1-infected and uninfected children. The Pediatrics Infectious Disease Journal, 18: 689 – 694.

UNAIDS, 2006. AIDS Epidemic Update, Joint United Nations Programme on HIV/AIDS. [Internet] [Geneva]. Available from: http://www.unaids.org/en/HIV_data/epi2006/ default.asp> [Accessed 15 February 2007].

UNAIDS, 2001. The Global Stragegy framework on HIV/AIDS [Internet] Geneva: Joint United Nations Programme on HIV/AIDS. [Internet]. Available from: http://data.unaids.org/publications/IRC-pub02/JC637-GlobalFramew_en.pdf>[Accessed 15 February 2007].

Van der Sande, M.A.B., Van der Loeff, M.F., Aveika, A.A., Sabally, S., Togu Sarge- Njie, R., Alabi, A.S., Jaye, A., Corrah, T. and Whittle, H.C. 2004. Body Mass Index at the time of HIV diagnosis: A strong and independent predictor of survival. Journal of Acquired Immune Deficiency Syndromes, 37: 1288 – 1294.

Van der Walt, E., Steenkamp, L. and Venter, D. 2004. Impact of nutrition support in children infected with and affected by HIV/AIDS (abstract). South African Journal of Clinical Nutrition, 17(2): (S26).

Wheeler, D.A., Gibert, C., Launer, C., Muurahainen, N., Elion, R., Abrams, D. and Bartsch, G. 1998. Weight loss as predictor of survival and disease progression in HIV infection. Journal of Aquired Immune Deficiency Syndromes and Human Retrovirology, 18(1): 80 – 85.

CHAPTER 2 – LITERATURE REVIEW

IMMUNE FUNCTION, NUTRITION AND HEALTH: DOES SUPPLEMENTATION OF CHILDREN LIVING WITH HIV MAKE A DIFFERENCE?

2.1 Introduction

AIDS was first recognised in 1981 and its causative agent - HIV-1 - was identified in 1983. HIV-2 causes similar symptoms to HIV-1, but is less aggressive and is found mainly in Western Africa. Eight HIV-1 subtypes exist, with a strong association between the subtype and mode of transmission. In Southern Africa, subtype C accounts for the majority of new infections in the heterosexual population. Since 1981, AIDS has grown to be the leading cause of death in Africa (Wood, 1999). Pediatric AIDS was first described in 1982. Since then, mother-to-child transmission has been dramatically reduced in developed countries by means of antiretroviral drugs (Miller, 2003). In Africa however, HIV-infection has reversed gains in child survival and lowered life expectancy (Cant et al., 2003, p. 1295).

In a review by Dabis and Ekpini (2002), it was shown that an estimated 26 to 45 percent, and 35 to 59 percent of African children infected with HIV, die by their first and second birthdays respectively. Only a fraction of them survive until the age of five years.

Large scale orphaning has traditionally been a problem associated with war, famine or disease. HIV/AIDS has transformed this issue into a chronic problem whose impact will only be felt in the next few decades. More than 15.6 million children have lost either a mother or father to AIDS or related causes worldwide (WHO, 2006). In South Africa, it is

estimated that 3 million children under the age of 15 might already be affected – either infected or lost one or both parents from HIV/AIDS (Woollard, 2003). These orphans are likely to be more vulnerable to malnutrition. This is without a doubt turning into one of the most pressing public health issues South Africans have to face.

During the first few years of the HIV pandemic, wasting syndrome was described as one of the major complications of the disease (Kotler, 1999). Wasting was subsequently included as one of the principal criteria for the diagnosis of symptomatic AIDS in both adults and children (CDC, 1994). According to Friis (2002, p.2), "since the antibiotic era, the importance of good nutrition as a fundamental principle in preventative and curative medicine seems to have lost hold, especially among health professionals." The World Health Organisation, however, has recently emphasised that the relationship between nutritional deficiencies and infections and the public health implications thereof has been clearly demonstrated (United Nations System, 2004).

After the introduction of antiretroviral drugs in the 1990s, there seems to be a growing perception amongst health professionals that HIV wasting is becoming less of a problem. This is not the case. As a number of patients lack access to antiretroviral therapies in African countries, as well as the presence of treatment failure in developed countries, AIDS related wasting, together with deterioration in immune function, has not disappeared. It is therefore still necessary to develop nutrition related strategies to prevent early weight loss and treat patients with malnutrition (Salomon et al., 2002).

In this chapter and as background, the researcher will review the following information regarding HIV-infection in children:

- Immune function and changes thereof as a result of malnutrition or HIV infection;
- HIV/AIDS in children (etiology and transmission, pathogenesis, epidemiology, diagnosis, manifestations, survival patterns, stages and the classification);
- Impact of HIV infection on the growth of children;
- The impact of HIV-infection on biochemical and metabolic parameters of children;
- Management of HIV-infected children (including nutrition supplementation strategies); and
- Nutrition intervention and the impact on immune function in children.

2.2 Immune function and changes thereof as a result of malnutrition or HIV infection

The term “immunity” refers to the manner in which the immune system responds to invading pathogens or foreign substances - either in a protective sense or in a harmful way. Most microorganisms are effectively dealt with by the immune system, thereby preventing disease (Hubley, 2002, p. 16).

2.2.1 The immune response

The immune response involves two mechanisms - **nonspecific and specific**.

Nonspecific mechanisms include the phagocytic and complement systems. They develop independently of the presence of infections and are not specific for any given infectious agent.

Specific mechanisms involve antibody-mediated and cell-mediated immunity (Sorensen et al., 2001, p. 55).

2.2.1.1 The innate immune response (nonspecific mechanism)

The first line of defense consists of physical barriers like the skin and mucosal surfaces (epithelial lining of the airways and gut), as well as phagocytic cells and the complement system. Phagocytic cells are specialised cells that engulf whole particles or release toxic substances, thereby killing the invading pathogen. The complement system, which entails activation of serum-proteins, facilitates phagocytosis and increases vascular permeability which enables the phagocytic cells to reach the site of infection. This response is also being called the ***innate*** immune response (Gray, 2005, p. 121).

The innate immune response is a primitive form of defense and does not appear to be qualitatively or quantitatively affected by repeated contact with the same stimulus (Powell et al., 2001, p. 70). Though minor bacterial infections (most extracellular pathogens) are normally terminated by these mechanisms, a more advanced response is needed to terminate some other infectious agents (Sorensen et al., 2001, p.55).

2.2.1.2 The acquired immune response (specific mechanism)

T and B-lymphocytes, which originate in the bone marrow and other lymphoid tissues, migrate to the peripheral lymphoid circulation when mature, and provide constant surveillance against invading pathogens. This process is also called the ***acquired*** immune response, and has amongst others, “specificity” and “memory” as characteristics, unlike the innate immune response (Gray 2005, p.120).

As the receptors of these B-cells and T-cells are specific to the antigens presented to them, binding of the antigen to the receptor cells leads to the generation of antigen specific immune mechanisms. While the B-cells produce antibodies which destroy invading pathogens before they enter the cell of the host, the T-cells control and regulate the immune response. On binding of an antigen to B-cell surface immunoglobulins, cell division takes place, followed by differentiation into antibody producing plasma cells (proteins). These secreted antibodies attach to surviving bacteria, creating the opportunity for phagocytosis. Furthermore, the antibodies direct and amplify the innate immune response by activating complement through an alternative pathway (Sorensen et al., 2001, p. 57).

Cell-mediated immunity is essential against intracellular pathogens, including viruses, mycobacteria, salmonella, fungi and protozoa, as they frequently survive within monocytes and macrophages and do not activate the alternative pathway of complement.

In response to the presence of these pathogens, T-helper cells ($CD4^+$ cells) primed by dendritic cells, proliferate and secrete lymphokines, which activate monocytes and macrophages, followed by phagocytosis.

T-suppressor cells ($CD8^+$ T-cells) develop into cytotoxic T-lymphocytes, also referred to as effector $CD8^+$ T-cells. The latter are capable of killing virally infected cells, thereby exposing the viruses from the lysed cells to the action of antibodies, complement and phagocytic cells (Cant et al., 2003, p. 1258; Sorensen et al., 2001, p. 55). In the process of eliminating the antigen, most of the killer $CD8^+$ T-cells will die through apoptosis. Only

a small amount of memory $CD8^+$ T-cells will re-circulate through the lymphatic blood circulation.

2.2.1.3 The importance of the CD4+ T-cell

The $CD4^+$ T-cell plays a central role in coordinating the acquired immune response. Depending on the type of pathogen invading the host, the $CD4^+$ T-cell will release cytokines which either stimulate the cytolytic $CD8^+$ T-cell response, or promote the antibody response. Depletion of these $CD4^+$ T-cells as in the case of HIV-1 infection will remove the most important role player from the acquired immune response and is one of the main reasons of the immune compromised state in HIV-infected individuals (Gray, 2005, p. 124).

2.2.2 Immune function in children

The newborn's immune system exhibits a physiological immunodeficiency in full term as well as pre-term. This deficiency is, however, more exaggerated in the premature and particularly evident in sick or stressed pre-term infants. Immunoglobulin-G (IgG) is transferred via the placenta and partially offsets the deficiency. The transfer of immunoglobulin, however, takes place late in gestation; therefore the pre-term infant has significantly reduced levels of immunoglobulins. Immunoglobulin production and specific antibody responses commence soon after birth. Initially only Immunoglobulin-M (IgM) is produced, but the IgG responses develop gradually until the age of two months when infants are able to produce good IgG antibody responses. All this happens while the maternal IgG falls due to catabolism. At the age of three to six months the infant's production picks up. The antibody levels thereafter rise at different rates; adult levels of

IgM are achieved at four to five years, IgG by seven to eight years while IgA levels only achieve adult values in teenagers (Cant et al., 2003, p. 1262).

Several sources (Sorenson et al., 2001, p.61; Nielsen, 1999) confirm that children have significantly higher absolute numbers and percentages of lymphocytes than adults. The T-lymphocyte responses are poor largely because of the high proportion of naïve T-lymphocytes which are more difficult to stimulate. T-lymphocyte immunity appears to mature rapidly in the first few weeks of life following antigen exposure. However, the differences in T-cell numbers and $CD4^+$: $CD8^+$ ratios between adults and children suggest that the maturation and development of the cell mediated immune response continues through early childhood (Cant et al., 2003, p. 1264).

2.2.3 The impact of malnutrition on immune function

For the last few decades health workers in underprivileged communities noted the mutually aggravating interaction between malnutrition and infection. This was very evident from studies in young children where the incidence of diarrhoea in wasted children was more than twice that of non-wasted children (Tomkins, 1981). According to a statement from Chandra (1983) more than 20 years ago, nutritional status is a critical determinant of mortality and morbidity in children. Malnutrition is still acknowledged to have devastating effects upon both the innate and acquired immune function (Beisel, 1996). PEM can cause widespread atrophy of the lymphoid tissues, especially in children. Affected areas include the thymus, spleen, tonsils and lymph nodes. Histological evidence confirms that the atrophy is the greatest in the T-lymphocyte areas of these tissues. PEM therefore causes a significant repression of cell mediated immunity and the function of T-lymphocytes.

Apart from the widespread atrophy of lymphoid tissues in non-HIV related malnutrition, malnourished children show a decreased $CD4^+$:$CD8^+$ (T-helper/T-suppressor cell) ratio, a diminished $CD4^+$ cell count, impaired delayed hypersensitivity and increased serum globulin concentrations. All these changes imply that the immune systems of malnourished children are not able to recognise and destroy invading pathogens or substances (Chandra, 1991). In contrast, general B-lymphocyte numbers and functions seem to be maintained. This immune suppression in malnourished children therefore diminishes the ability of the body effectively to deal with the challenge of infectious diseases (Beisel, 1996).

All immunological dysfunctions associated with malnutrition have been termed Nutritionally Acquired Immune Deficiency Syndrome (NAIDS). Infants and small children are at particular risk to develop NAIDS because their immune systems are immature and they have little protein reserves in their skeletal muscles. These features found in NAIDS strongly resemble the effects of HIV infection, but are fully reversible with nutritional rehabilitation (Beissel, 1996).

In support of the above, epidemiological studies of both adults and children infected with HIV suggest that the nutritional status can impact independently on quality of life and length of survival. This outcome is probably due to the effect of malnutrition on the immune function (Miller, 2003).

2.2.4 The impact of HIV infection on immune function

HIV is a retrovirus because it encodes the enzyme *reverse transcriptase*. The virus replicates itself by allowing a DNA copy to be made from viral RNA, going against what

is considered to be the normal flow of information (Figure 2.1). In that way, the virus is encrypted into the host's cells and in particular the cells that are supposed to fight the infection, namely the $CD4^+$ T-cells and macrophages. After initial transmission of HIV-1, the virus apparently "seeds" to lymphoid depots where it exits as a whole virus on the surface of the follicular dendritic cells (FDC) in the lymph node. The normal function of the FDC is then thought to be overridden which causes immune suppression in the host. This can often be measured by a depressed $CD4^+$ cell count. In turn, the depressed CD4 count is proportional to the immunopathology caused by the HIV infection (Gray, 2005, p. 130; Martin, 2002).

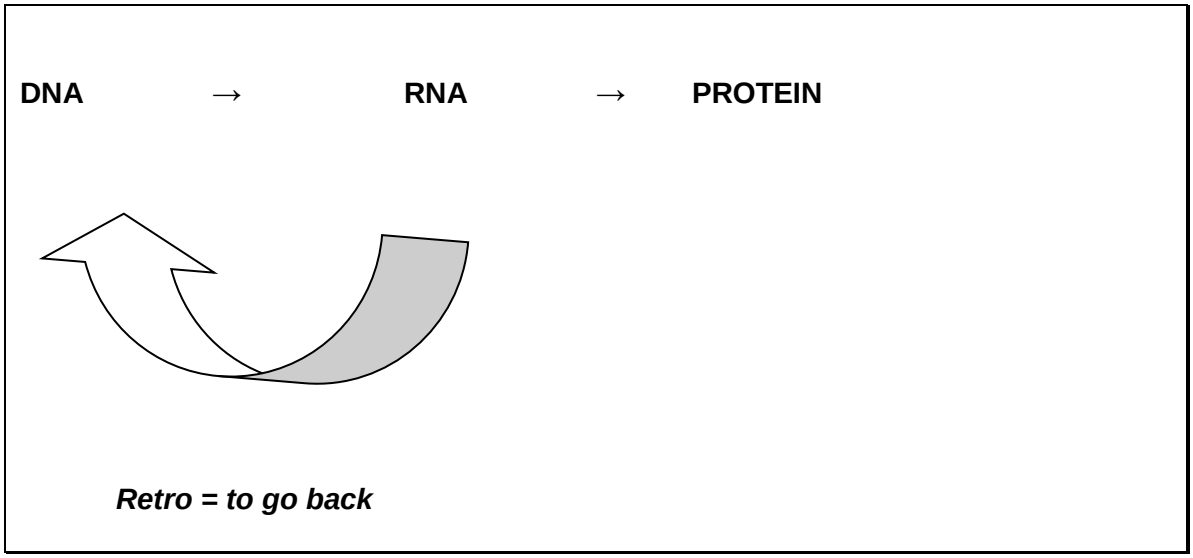

Figure 2.1: Schematic diagram of genetic flow of HIV-1 (Morris & Cilliers, 2005, p. 80)

The vigorous burst of viral replication following primary infection, results in very high levels of 10^5 to 10^7 HIV-RNA copies per millilitre. During the initial immune activity resulting from the HIV-infection, $CD8^+$ cytotoxic T-lymphocytes are thought to play an important role in the initial containment of the virus by slowing viral replication and disease progression. A "viral set-point" is normally reached within six to 12 months after

the initial infection, with levels of 10^2 to 10^6 HIV-RNA copies per millilitre. From animal models it has been demonstrated that individuals who rapidly progress to AIDS, do not control viral replication and have possibly never mounted effective immune responses. In the latter situation, there would be uncontrolled viral replication and a higher probability of disease progression (Gray, 2005, p. 130 – 131; Martin, 2000).

2.3 HIV/AIDS in children

HIV has a bimodal distribution of disease presentation and progresses more rapidly in children than in adults. In about 20 percent of children in developed countries, rapid disease progression will occur within the first two years of life. However, in the majority of children a more intermediate rate of progression will take place and they will tend only to develop severe immunosuppression by seven to eight years of age. A small group of HIV-infected children will remain healthy with normal to minimally decreased $CD4^+$ T-cells (Nielsen, 1999).

Several factors including access to antiretroviral drugs, prophylaxis against *Pheumocytis carinii* and better nutritional support may ensure that HIV-infected children are surviving longer with a better quality of life. In large parts of Africa, these interventions are not freely available (Bobat et al., 1999).

According to Cotton et al. (1998), pediatric health care services in sub-Saharan Africa are burdened by the increasing numbers of HIV-infected children and it is evident that the problem of providing optimal care and support will not disappear.

2.3.1 Etiology and transmission

The cause of AIDS is HIV. In HIV-infected individuals, the virus is present in blood, semen and other body fluids such as breast milk and saliva. Direct exposure of individuals to these fluids leads to a risk of contracting the infection. The risk is dependent on the integrity of the exposed site, the type and volume of the body fluid, and the viral load. The major modes of transmission are sexual, parenteral or vertically from mother to child (Chadwick & Yogev, 1995).

The acquisition of HIV by infants appears to be multifactorial. About 5 to 10 percent of new HIV-infections are in children and more of 90 percent of these occur in utero, intrapartum or via breastfeeding. An infant is considered to have in utero infection if virologic tests are positive within the first 48 hours of life. Intrapartum infection occurs when the infant is infected during delivery. In these cases, diagnostic tests are negative within the first 48 hours of life, but turn positive within one week after delivery (Nielsen, 1999). Infants with in utero infection, appear to have a more rapid disease course than those infants who acquired the infection intrapartum.

As summarised by Rabie et al. (2006) and Nielsen (1999), the risk of mother-to-child transmission (MTCT) is decreased by the following:

- A very low to undetectable maternal virus load and absence of other maternal infections;
- Use of antiretroviral therapy by the mother during pregnancy and in the perinatal period by exposed infants;
- High levels of maternal neutralising antibodies;

- Elective cesarean sections (instead of normal vaginal birth, especially with prolonged rupture of amniotic membranes);
- Normal birth weight, full term infants;
- In case of postpartum transmission of HIV by breastmilk, it was established that a 28 percent risk of infection has been documented when women seroconvert during lactation, opposed to only a 14 percent risk in the presence of an established HIV infection.

2.3.2 Pathogenesis

The almost uncontrolled spread of HIV-1 indicates that the virus effectively counteracts both innate and acquired immunity. This single stranded RNA retrovirus - from the *Lentivirus* genus - excels in taking advantage of cellular pathways, while neutralising and hiding from the different components of the immune system (Simon et al., 2006). Following mucosal exposure, HIV is transported to the lymph nodes via dentritic, $CD4^+$ T-cells or Langerhans cells, where the infection starts (Chadwick & Yogev, 1995).

The virus infects the $CD4^+$ T-cell in a complicated sequence of events involving initial attachment and receptor engagement through the viral gp120 (external glycoprotein) and gp41 (transmembrane protein) to the $CD4^+$ cell membrane (Figure 2.2). Having penetrated the cell, the viral core is released into the cell cytoplasm and a DNA copy is transcribed from the RNA genome by the reverse transcriptase (RT) enzyme that is carried by the infecting virion. Reverse transcription is an error prone process, and multiple mutations arise with ongoing replication (Simon et al., 2006).

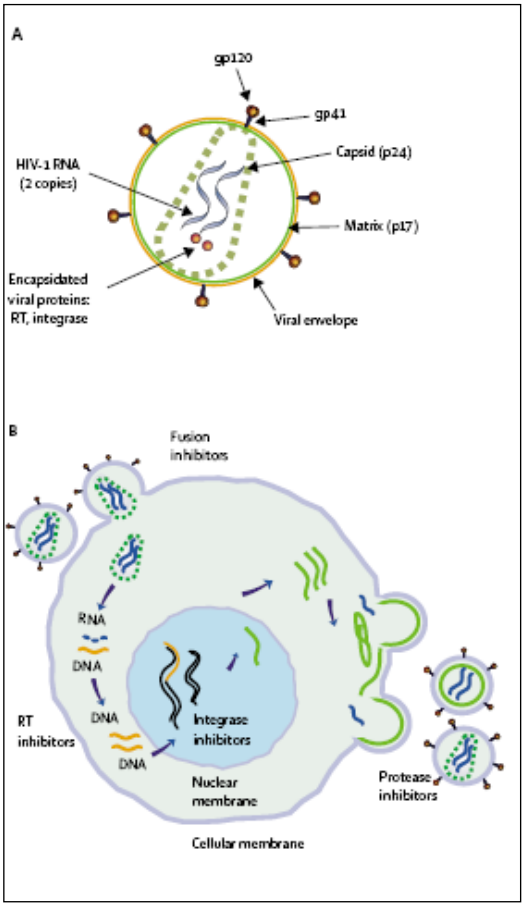

Figure 2.2: Process by which the HIV-1 retrovirus encodes the structural genes (Simon et al., 2006)

On host cell activation, this DNA copy is used as a template to transcribe new RNA copies. New viral structural proteins and viral enzymes, such as protease and reverse transcriptase, are subsequently formed, which migrate to the cell surface to produce infectious viral particles. These particles bud from the cell surface, incorporating the host cell membrane as their own lipid bilayer coat and cell lysis occurs. The new infectious virus (virion) is then available to infect uninfected cells and starts the whole process all over again. It has been calculated that 10^{10} virions are produced on a daily basis, while 10^{9} $CD4^{+}$ cells are destroyed. This represents a turnover of 30 percent of total viral

burden and six to seven percent of the total body $CD4^+$ cells daily (Simon. et al., 2006; Martin, 2002).

As $CD4^+$ cells are pivotal in orchestrating the immune response - as described in 2.2.1.3 - any depletion in numbers renders the body susceptible to opportunistic infections and oncogenic virus-related tumours. The predominant opportunistic infections in HIV disease are intracellular parasites (*Mycobacterium tuberculosis*) or pathogens susceptible to cell mediated, rather than antibody mediated, immune responses. ***The reduction in the number of $CD4^+$ cells circulating in the peripheral blood is closely correlated with the amount of plasma viral load and these two indicators are normally used as measures of disease progression.***

Virus specific $CD8^+$ cytotoxic T-cell lymphocytes develop rapidly after infection and are the most important mechanism in recognising, binding and lysing infected $CD4^+$ cells. They play a crucial role in controlling HIV replication after infection and determine the viral set-point and subsequent rate of disease progression. Perinatally infected infants in general display a shorter period of clinical latency than adults. Intrauterine infection can coincide with a period of rapid expansion of $CD4^+$ cells in the foetus, which could effectively infect the majority of the body's immune competent cells. The normal migration of these cells to lymphoid tissues would result in systematic delivery of HIV, unchecked by the immature immune system of the foetus. The infection will therefore be established before normal development of the immune system is complete (Chadwick & Yogev, 1995).

A rapid rise in viral load to a few hundred thousand, within one to two months, can be observed in infants. The subsequent slow decline, which contrasts sharply with the rapid

decline of the viral load in adults after primary HIV-1 infection, suggests that the infant's still immature immune system has difficulty containing the viral infection (Shearer et al., 1997). Infants infected during delivery are more likely than those infected in utero, to have lymphoid hyperplasia and to survive longer.

In infants, HIV-related immunosuppression is imposed on a still developing immune function. The HIV disease tends to be more aggressive than in adults, which leads to a range of unique problems.

2.3.3 Epidemiology

The HIV transmission rate in South Africa can be linked to immediate and underlying causes. Immediate determinants include behavioral variables like sexual networking patterns and sexual practices, as well as biological variables including the high prevalence of sexually transmitted disease and male circumcision. Medical factors, including the availability of antiretroviral drugs and treatment of AIDS-related opportunistic infections, can also influence the spread of HIV (Gouws & Karim, 2005, p. 50).

Underlying determinants include socio-economic factors like poverty, the migrant labour system, the practice of commercial sex, low status of women and illiteracy (Allen et al., 2000). In 2002 an estimated number of 3.2 million children worldwide, under the age of 15 years, were living with HIV/AIDS. Of those, 800 000 were newly infected and 610 000 died (Bateman, 2004; UNAIDS, 2002). At present an estimated 80 000 HIV-infected children are born in SA annually (Department of Health, 2003) and 1500 HIV-infected children in sub-Saharan Africa per day (WHO, 2006).

Accurate estimates of the number of children in Africa infected with the HI-virus are not available, because very little random-sample surveys included children and the overwhelming majority of children are never tested for HIV (Gisselquist et al., 2004).

In the absence of representative paediatric seroprevalence surveys, the WHO and UNAIDS estimate the number of paediatric HIV infections from:

- The HIV prevalence in pregnant women;
- Observed rates of vertical transmission; and
- Assumed paediatric survival with HIV.

In Africa, vertical transmission is assumed to infect 35 percent of children born to HIV infected mothers. Fifty percent of these children are assumed to survive until two years of age and 40 percent until five years of age. Taking into account these parameters and assumptions, HIV prevalence in children at birth would be roughly one fifth of the HIV prevalence in mothers – dropping in children older than a year, due to higher mortality in children (UNAIDS/WHO, 2002). From this it can be assumed that approximately nine percent of the total prevalent infections in Africa, have been in children.

More information regarding tendencies can be obtained by looking at the HIV prevalence in paediatric in-patients, for instance at Chris Hani Baragwanath Hospital. Increases from 7.1 percent in 1992 to 42 percent in 1996 have been observed (Gisselquist et al., 2004) in the prevalence of HIV-infection in children.

2.3.4 Diagnosis, manifestations, survival patterns, stages and classification

2.3.4.1 Diagnosis

The definite diagnosis of HIV infection of children requires diagnostic testing that confirms the presence of the HI-virus. Antibody testing confirms only HIV antibodies. The maternal HIV antibody which is transferred passively during pregnancy, can persist for up to 18 months in the infant. The HIV antibody test results should therefore be confirmed with virological tests, such as assays, to detect HIV DNA, HIV RNA or p24 antigen in younger children (WHO, 2006; Rabie et al., 2006; Martin, 2000).

2.3.4.2 Manifestations

The first reports describing the manifestations of perinatally acquired HIV infection, were primarily retrospective studies of children who had early onset of symptoms. These reports were therefore biased to a more rapidly progressive disease. Data from longitudinal studies indicate a bimodal distribution of disease progression, with 20 to 30 percent of children developing a profound immune deficiency before one year of age, with the remainder experiencing a slower disease progression (Chadwick & Yogev, 1995). An early European study, before antiretrovirals were freely available to children, has shown that more than 60 percent of infected children experienced HIV related signs or symptoms by the age of 9 months, and about 75 percent by 16 months of age (Gisselquist et al., 2004).

Common clinical manifestations in HIV infected children (mean age of four years) in India, included oral candidiasis (43%), pulmonary tuberculosis (35%), recurrent

respiratory infections (26%), bacterial skin infections (21%), papulo-pruritis dermatitis (19%), hepatosplenomegaly and lymphadenopathy (14%) and chronic diarrhoea (7%) (Madhivanan et al., 2003).

In general, the presence of opportunistic infection, progressive encephalopathy or hypogammaglobulinaemia at any age carries a poor prognosis. Generalised lymphadenopathy, hepatosplenomegaly, lymphoid interstitial pneumonitis, parotitis, HIV dermatitis and recurrent bacterial infections are associated with a more favorable prognosis (Chatwick & Yogev, 1995). HIV has a large variety of clinical manifestations in children; these will be described in more detail in 2.3.4.4 as part of the classification.

2.3.4.3 Survival patterns

In a large cohort of children who were perinatally exposed to HIV infection, patterns of mortality indicated that HIV-infected children were nine times more likely to die than uninfected children, despite the fact that these children represented the “less rapid progressors”. The most common reported causes of death were conditions suggestive of paediatric AIDS (Taha et al., 1999).

In South Africa (North West province) 61.9 percent of deaths in children below five years of age were recently shown to be AIDS or HIV related (Krug et al., 2004). Another South African study has demonstrated that a mortality of 35.4 percent occurred within the first year of life (mean age: 10 months). Most of these children presented with diarrhoea, pneumonia, failure to thrive or marasmus, and severe thrush at the time of death. Half of the children experienced neurological abnormalities. These clinical findings often presaged rapid deterioration and death (Bobat et al., 1999).

Survival data of HIV-infected children from Cape Town indicate a mean survival of only 32 months (Hussey et al., 1998). In a recent publication by the WHO (2006), it is concluded that an estimated third of HIV-infected children will have died by one year of age and half by two years of age. Most researchers now agree that strategies, which should include nutrition support, need to be implemented and monitored in order to improve quality and duration of life in HIV-infected children.

2.3.4.4 Stages and classification

To better characterise the stages and degree of immunologic and clinical involvement of the HIV-infected child, the CDC developed a revised paediatric HIV classification system, which stratifies patients according to **immunologic categories** based on age-specific $CD4^+$ cell counts (Table 2.1).

Table 2.1: 1994 Revised Paediatric HIV Classification System: Immunologic Categories Based on Age-Specific $CD4^+$ Lymphocyte Count and %(CDC - MMRW, 1994)

	Age of child		
Immune category	**</= 12 months**	**1-5 years**	**6-12 years**
	Cells/mm^3 (%)	**Cells/mm^3 (%)**	**Cells/mm^3 (%)**
Category 1: No suppression	>/= 1500/ > (25)	>/= 1000/ > (25)	>/= 500/ > (25)
Category 2: Moderate suppression	750-1499 (15 - 24)	500- 999 (15 - 24)	200-499 (15 - 24)
Category 3: Severe suppression	< 750 (<15)	< 500 (< 15)	< 200 (< 15)

The WHO (2006) has recently published a revised **clinical** staging classification for children with HIV. According to this staging (Table 2.2), children can be classified into stages 1 to 4 according to clinical signs and conditions where confirmatory diagnostic testing is required.

Table 2.2: Clinical staging according to the WHO (2006)

Stage 1:

- Asymptomatic
- Persistent generalised lymphadenopathy

Stage 2:

- Hepatosplenomegaly
- Papular priuritic eruptions, seborrhoeic dermatitis
- Extensive human papilloma virus infection, molluscum contagiosum
- Herpes Zoster
- Fungal nail infections
- Parotid enlargement
- Recurrent oral ulcerations, lineal gingival erythema, angular cheilitis
- Recurrent or chronic respiratory tract infections (otitis media, otorhoea, sinusitis)

Stage 3:

- Moderate unexplained malnutrition
- Unexplained persistent diarrhoea
- Unexplained persistent fever
- Oral candidiasis
- Oral hairy leukoplakia, necrotising ulcerative gingivitis or periodontitis
- Severe recurrent bacterial pneumonia
- Pulmonary TB

Stage 4:

- Unexplained severe wasting or severe malnutrition
- Pneumocystis pneumonia
- Extrapulmonary TB
- Oesophageal candidiasis
- Recurrent severe bacterial infections
- Chronic herpes simplex
- Karposi sarcoma
- Central nervous system toxoplasmosis
- HIV-associated encephalopathy

While the clinical **categories** that define stages of paediatric and adult HIV infection are similar, the clinical **manifestations** in children can differ a lot from those in adults. Children are prone to develop opportunistic infections at much higher $CD4^+$ cell counts than do adults and they also have a different pattern of clinical manifestations, including (Hanna, 1996):

- A higher rate of recurrent and serious bacterial infections (*Pneumocystis carinii* pneumonia (PCP) is sometimes diagnosed before the HIV-infection) ;
- Developmental problems including failure to grow and thrive which occur earlier in children than wasting in adults;
- A more likely chance to have enlarged livers, spleens and lymph nodes;
- A high prevalence of lymphoid interstitial pneumonitis (LIP), uncommon in adults;
- Early onset of central nervous system abnormalities versus late onset in adults;
- Low occurrence of malignancies, which is common in adults;
- Parotitis;
- Chronic bilateral otitis media and thrush - even in children without AIDS.

The disease spectrum in HIV-infected children has changed dramatically over the last decade. Especially in the developed world, children have benefited from achievements regarding new drugs, vaccination, a decrease in perinatal transmission rates and nutrition support. The challenge for developing countries would be to achieve similar success in managing the different stages of HIV infection (Oleske et al., 1996).

2.3.5 Markers of immune function to evaluate disease progression

2.3.5.1 Viral load versus CD4$^+$ cell count – which one is the better indicator?

Viral load measurements, together with CD counts, represent the current standard of care to monitor patients with HIV infection (Martin, 2002). The measurement of the viral load is expressed as HIV-1 RNA copies per milliliter of plasma and recent data have shown that viral load estimations are a better marker of clinical outcome than CD4$^+$ cell counts (Martin, 2000).

In a study by Mellors et al. (1996), it was demonstrated that changes in plasma HIV-RNA levels and CD4$^+$ counts are both independent predictors of disease progression; however, plasma viral load was a better predictor of progression to AIDS and death than the number of CD4$^+$ T cells. In two separate studies it was shown that a 2-fold (0.3 log) and 3-fold (0.5 log) decrease in HIV-RNA levels were associated with a 27 percent and 63 percent reduction in the relative hazard of disease progression respectively (Coombs et al., 1996; O'Brien et al., 1996). Another study indicated that a 1-log treatment-induced decrease in viral load was associated with an 80 percent decrease in the risk of disease progression and was a more powerful predictor of clinical outcome than CD4$^+$ cell counts. In a recent meta-analysis of studies that evaluated treatment-mediated changes in CD4$^+$ cell counts and viral loads as indicators for disease progression, a linear relationship between these markers and the risk of progression was demonstrated. An observed reduction of 0.18 log in viral load, related to an increase in 30 percent in the time it took to progress to AIDS (Fawzi et al., 2005).

Quantitative measurement of plasma-1 RNA can be expressed as:

- The number of HIV-1 RNA copies/ml of plasma; or
- The logarithmic equivalent – $\log_{10}$ equivalent (a 1-log change represents a 10-fold change).

Biological variability, together with intra-assay variability, accounts for approximately 0.5 $\log_{10}$ copies/ml change (3-fold variation) between measurements. A greater than 3-fold difference therefore assumes significance, whereas in a clinical context a 10-fold change would be regarded as clinically significant (Martin, 2002). It is important to realise that viral load estimations in small infants are less reproducible than in adults as the spontaneous variation is larger (Martin, 2000).

2.3.5.2 Total lymphocyte count and $CD4^+$ cell count: Absolute values versus CD4%

The total lymphocyte count (TLC) and $CD4^+$ cell counts in healthy infants are considerably higher than those of uninfected adults and only decline to adult values by the age of six years. The $CD4^+$ percentage (of the TLC) varies less with age and in children less than five years of age, is thought to be more valuable than $CD4^+$ cell count. Values are also influenced by intercurrent illness, physiological changes and test variability.

Threshold $CD4^+$ percentage levels to indicate severe immunodeficiency is as follows:

- < 25 percent for infants up to 11 months;
- < 20 percent for children aged 12 – 35 months;
- < 15 percent for children aged three years and above.

According to the WHO (2006) these values correspond to a 12-month mortality risk of five percent or less. This data is however derived from HIV-infected children in resource-rich countries and similar data is not available for children in developing countries.

As in the case of adults, the TLC also significantly predicts the mortality risk in HIV-infected children. As with the $CD4^+$ count, the predictive value of TLC in small infants under six months of age is poor, as high mortality can occur even at high TLC values. Severe immunodeficiency is indicated by the following TLC values:

- < 4000 cells/mm^3 for infants up to 11 months;
- < 3000 cells/mm^3 for children aged 12 – 35 months;
- < 2500 cells/mm^3 for children aged three to five;
- < 2000 cells/mm^3 for children aged five to eight (WHO, 2006).

2.3.5.3 The importance of $CD8^+$ cell counts

Very little data are available in regard to $CD8^+$ cell counts in HIV-infected children. In one study (Italian Register for HIV Infection in Children, 1994), it was demonstrated that “long term survivors” had higher $CD8^+$ cell counts than short term survivors or uninfected children. It was deducted that the $CD8^+$ counts in these long term survivors were higher at birth. $CD8^+$ cells can slow the HIV infection down through cytotoxicity to infected cells and suppression of viral replication. $CD8^+$ counts then proceed to decline in a linear way every year, just as can be observed from $CD4^+$ cell counts.

2.3.5.4 Summary

When the abnormalities as described in 2.3 appear in HIV infected children, it becomes difficult to decide whether it is due to the infection with the virus, or to the development of malnutrition. The possibility exists that nutritional rehabilitation may have a general, non-HIV-related, beneficial effect on immune response (Guarino et al., 2002). These effects may be more pronounced in developing countries where a large number of children do not have access to antiretroviral drugs.

2.4 The impact of HIV infection on the growth of children

2.4.1 Introduction

If the role of nutrition in HIV/AIDS gets scrutinised, it appears that one of the greatest quality of life-limiting complications that HIV-infected children face is the failure to gain weight (Oleske et al., 1996). Despite the fact that weight and height changes in children are often gross measurements, it is a fast and uncomplicated way to assess nutritional status. Weight loss in children should be considered much more seriously than the weight loss observed in adults, as constant growth is expected in children. No weight gain with constant linear growth can be considered a physiologic weight loss, since the child's weight for height is declining (Heller, 2000).

Below standard height-for-age z-scores (HAZ) or stunting, is a common finding in children with HIV or AIDS. A variety of disturbed growth patterns have been described for HIV-infected children (Steenkamp et al., 2004; Carey et al., 1998; McKinney et al.,

1993). The differences in growth patterns will very likely depend on the variable manifestations of the disease, the viral load and the study population.

Growth disturbances (Table 2.3) in HIV-infected children are normally detectable well before the onset of opportunistic infections or other manifestations. Growth faltering was observed by the age of three to four months from data from the United States, Europe and Africa (Arpadi, 2005). Growth stunting can occur in two ways; first a gradual decrease in weight growth velocity, followed by a decline in height growth velocity resulting in chronic undernutrition. The preservation of near normal weight-for-height z-scores (WHZ) is the child's way to adapt to long-term undernutrition. In other HIV-infected children, abnormalities in height growth velocity precede weight changes and may reflect altered endocrine function, possibly including deficient growth hormone, which is needed for normal skeletal growth (Miller, 2003). Progressive stunting is more typical than wasting in postnatal growth (Arpadi, 2005). Only one study reports growth beyond the age of two years where the effects of HIV on growth are diminished compared with earlier in life (Lepage et al., 1996). This finding may, however, reflect the early mortality of the most severely affected.

Table 2.3: Growth and nutritional changes in HIV infection (Hussey & Eley, 1998)

• Mean birth weight lower in infants of seropositive than seronegative mothers
• Growth parameters at birth similar for infected and uninfected infants born to seropositive mothers
• Mean birth weight lower in infants born to mothers with AIDS than to seropositive mothers without AIDS
• Postnatal growth is significantly impaired from as early as three months of age.
• Postnatal growth is compromised throughout childhood
• Preferential muscle wasting over fat wasting occurs.
• Somatic and visceral protein status depletion
• Essential amino acid depletion
• Multiple micronutrient deficiencies

Because the growth failure in children has been shown to be a predictor of disease progression (Chantry et al., 2003), it needs to be described in more detail.

2.4.2 International data

The variety of disturbed growth patterns that have been observed in HIV-infected children differed from symmetrical delays in weight and height to severe wasting with normal height. In an earlier study on 62 HIV-infected children in a developed country (McKinney et al., 1993), it was demonstrated that the weight-for-age z-scores (WAZ) of HIV-infected children was normally one standard deviation (SD) lower than those of uninfected children, while the height-for-age was more than 1 SD lower than uninfected children. Weight-for-length was basically similar in the infected and uninfected groups. It could therefore be deduced that the HIV+ children were proportionally smaller than the uninfected children in both length and weight, despite the fact that 73 percent of the HIV+ children received antiretroviral drugs. These data were supported by a study from Moye et al. (1996), where it was shown that HIV-infected children had a mean WAZ of 0.6 SD lower and a mean HAZ of 0.73 SD lower than non-infected children.

In a study by Henderson et al. (1998), on 42 HIV-infected children, it was demonstrated that the resting energy expenditure (REE) per kilogram bodyweight was significantly lower than those in a group of non-infected children. The REE was also negatively associated with the weight-for-age z-scores (WAZ) in the HIV-infected children. This observation is supported by research from Macallan (1998) in adults where it is shown that the total energy expenditure (TEE) in weight-losing HIV-infected individuals was lower, and that the weight loss was linked to a decreased food intake and not to an increase in the REE as previously thought (Kotler et al., 1990). Macallan (1998) also

demonstrated that there was no correlation between changes in REE and the rate of weight change and that the increased REE found in patients in earlier studies (Melchior et al., 1991) might be of little clinical importance. According to Arpadi et al. (2000), it was demonstrated that HIV-infected children presented with decreased REE and TEE. The only difference between the 16 HIV-infected children presenting with growth failure and the 26 with normal growth velocity was the fact that the ones with growth failure had a more significant deficit in energy intake than the latter group. It is thought that the decreased REE observed in HIV-infected children, could be attributed to the lower fat-free mass observed in these children as well as possibly an adaptation to a decreased energy intake.

From a large cohort of 1338 HIV-infected children between the ages of three and 15 years in the United States, it was demonstrated that approximately 30 percent experienced height growth retardation and 20 percent weight growth retardation (Carey et al., 1998). These figures seem to be lower than the growth retardation observed in HIV-infected children in developing countries.

2.4.3 South African data

Results from a few South African based studies (Steenkamp et al., 2004; Eley et al., 2002a; Eley & Hussey, 1999) amongst HIV-infected children, indicate that underweight occurs in approximately 25 percent and stunting in 55 to 60 percent (Figure 2.3). Hussey et al. (1998), demonstrated figures in a group of 193 symptomatic HIV-infected children in Cape Town, where only 7.4 percent had a WAZ above the third percentile. These figures are much worse than the international figures reflected in 2.4.2. The trend, however, reflects international data, which indicates that chronic malnutrition in the form

of stunting is a much bigger problem in the majority of HIV-infected children older than two years, than acute malnutrition in the form of severe wasting.

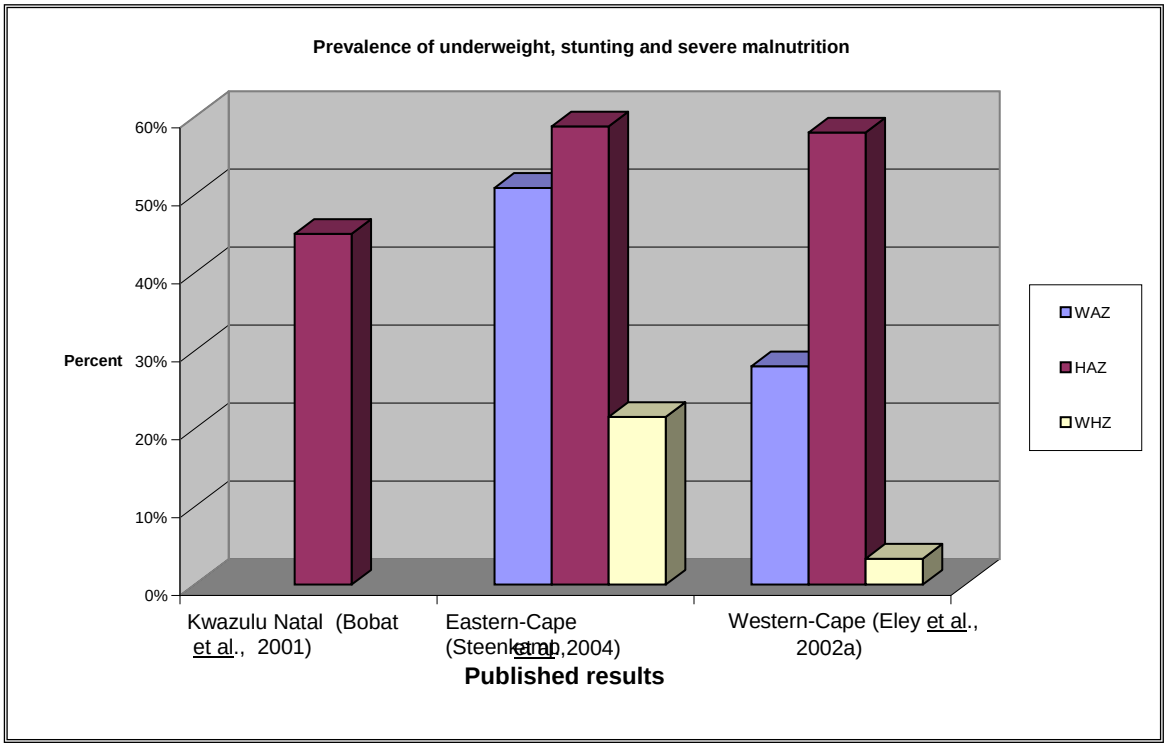

Figure 2.3: Nutritional status data in HIV-infected children – South Africa

2.4.4 The link between growth and disease progression

The relationship between protein-energy malnutrition and the associated adverse effects on the immune system is well recognised (2.2.3). The presence of protein-energy malnutrition may exacerbate the immunologic effects of the HI-virus. In both adults and children, wasting is related to the length of survival (Carey et al., 1998). According to Berhane et al. (1997), HIV-infected infants with growth failure have as much as a fivefold increase in the risk of early death. Weight loss has also been associated with an increase in infectious complications in patients with AIDS. The latter have been

associated with an increase in nutritional disorders (2.4.5), which would in turn aggravate the weight loss (Miller, 2003).

A higher HIV-1 viral load has been associated with a greater risk of growth failure and specific poor linear growth, in children (Pollack et al., 1997). Results from a study by Arpadi et al. (2000), indicated that viral replication played a central role in poor growth in HIV-infected children, when it was demonstrated that the growth rate was inversely related to the level of viral replication. Although a decreased energy intake also played a role in the development of growth failure, results indicated that viral replication was the more important factor. Other factors such as a lower $CD4^+$ cell count, infectious complications, maternal drug use during pregnancy and exposure to antiretroviral drugs (non-protease inhibitors) have been associated with growth problems (Miller, 2003).

Results from nutrition intervention studies in HIV-infected children indicate that increase in HAZ are associated with a reduced risk of subsequent clinical progression and subsequent immune reconstitution and is also weakly associated with declines in HIV RNA levels (Benjamin et al., 2003).

2.4.5 Causes of growth failure in HIV-infected children

The development of growth failure and malnutrition in HIV-infected children may be attributed to several mechanisms working either independently or synergistically (Kotler, 1999). It can be caused by recognisable illnesses and secondary infections that accompany HIV infection. Secondary causes which are potentially preventable can also be involved and these include decreased nutritional intake, diarrhoeal illnesses and anaemia (Arpadi, 2005). The causes are summarised in more detail in Table 2.4.

Table 2.4: Causes of malnutrition and wasting in HIV/AIDS (Miller, 2003)

Decreased nutrient intake
Anorexia
Peptic disease
Opportunistic infections of upper gastrointestinal tract
Idiopathic apthous ulcers
Dysgeusia
Pancreatic / hepatobiliary disease
Encephalopathy

Gastrointestinal malabsorption
Mucosal disease
- Infectious
- Inflammatory
- Disaccharidase deficiency
- Protein-losing enteropathy
- Fat malabsorption

Hepatobiliary
- Sclerosing cholangitis
- Chronic pancreatitis
- Co-infection with hepatitis B virus / hepatitis C virus

Increased nutritional requirements or catabolism
Protein wasting
Hypermetabolism in selected patients
Futile metabolic cycling
Secondary to:
- Fever, infection, sepsis
- Neoplasms
- Medications
- Release of cytokines or tumor necrosis factor

Psychosocial factors
Poverty
Illness in biological family members / orphans
Limited access to health care
Substance abuse

Inadequate nutritional intake plays a major role in the development of wasting. While reductions in food intake may develop directly as a result of painful sores in the mouth, pharynx or esophagus, other psychosocial factors may also affect appetite and food intake. In developing countries, the economic factors that influence food availability, as

well as the quality of the diet, also play an important role (Piwoz & Preble, 2000; Kotler, 1999).

Apart from the anorexia that develops as a result from the HIV infection, gastrointestinal malabsorption can further contribute to anorexia due to odynophagia, dysphagia, and abdominal pain associated with eating. Miller et al. (1997) showed that 70 percent of HIV-infected children revealed abnormal histology after gastrointestinal endoscopies. These mucosal changes might be due to local HIV-1 infection of the gut or secondary enteric infections. A large percentage of infected children therefore suffer from gastrointestinal pathology, resulting in chronic diarrhoea and malabsorption. The extent of malabsorption does not always correlate with the degree of malnutrition (Miller et al., 1991).

Acute diarrhoea is usually caused by either an intestinal infection or medication, while chronic diarrhoea often indicates lactose or fat intolerance. If the latter is unlikely in event of a low-fat, lactose-free diet, treatment of potential pathogens might be necessary (Heller, 2000).

Opportunistic infections may also affect the hepatobiliary system and pancreas, which can aggravate malabsorption. Small-bowel bacterial overgrowth, resulting from gastrointestinal dysmotility or hypochlorhydria, may also predispose the child to malabsorption (Miller, 2003).

Although different opinions (Miller, 2003; Johann-Liang et al., 2000; Henderson et al., 1998) are held by researchers regarding an increase or decrease in metabolic rates in HIV-infected children, all agree on the impact of cachexia on the development of wasting

(Miller, 2003; Preble & Piwoz, 2000). Cachexia is characterised by a significant loss of lean body weight as a result of metabolic changes that occur during the acute phase response to infective conditions – in this case, HIV infection (Kotler, 1999).

Psychosocial factors, like an unstable home environment with limited care and support from family members can also be important contributors to suboptimal nutrition and the development of growth failure in HIV-infected children (Miller, 2003).

2.4.6 The impact of ARVs on growth in children

According to literature (WHO, 2006; Visser, 2005, p. 463), increases in body weight and growth in children are linked with the introduction of highly active antiretroviral therapy (HAART). However, most studies indicate that improvements in weight and growth are linked to increases in the fat mass and not lean body mass, and that the improvement in weight is associated with hyperlipidaemia as well.

Despite the availability of ARVs in some parts of developing countries, it has become clear that nutrition will have to be addressed. From focus group discussions after the initiation of ARVs in Nairobi, most participants stated that the lack of food is the most likely cause of nonadherence to drug therapy. These researchers (Marston & De Cock; 2004) commented as follows: ”There truly is an irony, not captured in the language of treatment advocacy, in providing antiretroviral drugs to populations that lack access to safe water and food.”

2.5 The impact of HIV infection on biochemical and metabolic parameters in children

2.5.1 Introduction

Even in the presence of normal nutrition and asymptomatic HIV in developed countries, marginal and subclinical micronutrient deficiencies and changes in metabolic parameters may occur (Periquet et al., 1995). Deficiencies become more pronounced in patients with advanced disease in developing countries, where the diets available are inadequate to meet macro- and micronutrient needs of the community (Faber et al., 2001). Micronutrient deficiencies, resulting from the causes described in 2.4.5, can further compromise the immune system, resulting in poorer outcome (Steenkamp & Visser, 2002). Especially in Africa, deficiencies of a number of these micronutrients are more prevalent among children infected with HIV, than in HIV-negative children (Fawzi & Villamor, 2001). Other than vitamin A, no randomised trials examined the efficacy of micronutrient supplementation of children born to HIV-infected mothers (Tomkins, 2005; Fawzi, 2003).

As summarised by Long & Santos (1999), extensive research regarding the role of micronutrient supplementation in child health and survival in developing countries, has established that supplementation of children's diets with specific micronutrients can produce reductions in morbidity and mortality. Limited data are, however, available regarding nutrition intervention in HIV-infected children.

2.5.2 Micronutrients and disease progression: the impact of levels and supplementation

Micronutrient deficiencies compromise host immunity through a number of mechanisms. In addition to the direct effects on the immune system, anti-oxidant deficiencies can cause increased oxidative stress which leads to apoptosis of T-cells and in an indirect way compromises cell-mediated immunity (Friis & Michaelsen, 1998). The impact of micronutrient deficiencies on HIV disease progression has therefore been an area of great interest to researchers (Macallan, 1999).

Most studies have evaluated either the impact of micronutrient deficiency states or deficient dietary intake on disease progression and outcome and these are summarised in Tables 2.5 and 2.6 respectively. It is, however, important to remember that plasma markers of most of these micronutrients should be treated with caution, as they are affected by the acute-phase response and thus are susceptible to changes in binding proteins (Macallan, 1999). Serum zinc and retinol levels decrease during acute phase response, while serum levels of ferritin increase. If no information about concurrent infections is available, serum levels of micronutrients may not accurately reflect true micronutrient status (Tang et al., 2005).

Table 2.5: Relationship between micronutrient levels and disease progression

Plasma concentration	Impact on immune markers/rate of progression
Low selenium	Children had six time greater risk of mortality. Strongly associated with decreased $CD4^+$ cell counts and increased viral load and mortality in two studies. Also linked to lower WAZ scores. Levels inversely associated with risk of all-cause mortality (Kupka et al., 2005; Campa et al., 1999; Garland & Fawzi, 1999; Rodriguez et al., 1998; Miller et al., 1993).
Glutathione peroxidase	Several groups demonstrated depleted levels in HIV patients – also associated with decreased survival (Allard, 2002, p.61).
Low retinol	Increased mortality in adults and children (Piwoz & Preble, 2000; Semba & Tang, 1999; Semba et al., 1995)
Low vitamin A, B12, zinc	Significant decrease in $CD4^+$ cell counts; Low zinc levels correlated with disease progression in adults (Tang et al. 1996; Baum et al., 1995); low plasma levels of zinc not linked to disease progression in children (Henderson et al., 1997)
High vitamin E	Reduced rate of progression in adults(Tang et al., 1997)
Low vitamin E	Lower levels reported in HIV-infected children than in HIV-negative controls despite vitamin E supplementation (Mastroiacovo et al., 1996); Other studies no difference in levels despite HIV status (Omene et al., 1996)
Vitamin D	Severely deficient levels demonstrated in HIV-infected subjects with advanced disease (Haug et al., 1994).

Table 2.6: Relationship between micronutrient intake and disease progression

Nutrient	Impact on immune markers/rate of progression
Selenium	Supplementation may reduce impact of oxidative stress on HIV disease (Delmas-Beauviex et al., 1996)
Glutathione	Levels increased in HIV-infected adults after selenium supplementation. No effects were noted in terms of $CD4^+$ count changes (Delmas-Beauvieux et al., 1996).
Vitamin A	Reduced AIDS related mortality and diarrhoeal disease morbidity demonstrated in children. Increased $CD4^+$ counts (n=36) (Fawzi et al., 1999; Coutsoudis et al., 1995; Hussey et al., 1995); Increased mortality in PCR negative breast-fed infants (Humphrey et al., 2006); Vitamin A stores improved with vitamin A supplementation – even in the presence of severe diarrhoea in children with unknown HIV-status (Rollins et al., 2000).
Zinc	Data incomplete and inconclusive in children. Body weight and lymphocyte counts might improve and opportunistic infections decrease in adults (Reich & Church, 1994)
High vitamin E	Reduced rate of progression (Tang et al., 1997) and reduction of oxidative stress in HIV-infected adults (Allard et al., 1998); Supplementation of 1.5 mg vitamin E daily did not improve vitamin E levels in HIV-negative children to those of HIV-negative controls (Mastroiacovo et al., 1996)
Vitamin D	No intervention studies available.
Multivitamins	Supplementation decreased disease progression in HIV-infected pregnant women (n=1078) (Fawzi et al., 2004); HIV-infected children had more episodes of hospitalisation and diarrhoeal disease when supplemented with multivitamins than those supplemented with only zinc (Hussey et al., 2005)

2.5.2.1 Micronutrients with antioxidant properties

i) Selenium and glutathione peroxidase: Powerful antioxidants

Selenium is an element with similarities to sulphur. Selenium is required for lymphocyte proliferation and natural killer cell activity. It naturally forms compounds, of which some are incorporated into the biologically active selenoproteins, i.e. proteins that contain selenocysteine at the active site. One of the most important selenoproteins is the family of glutathione peroxidase, with important antioxidant capacity (Friis et al., 2002, pp. 184 - 186). The concentration of selenium in plasma, serum or whole blood is the most widely used static measure of selenium status. Plasma selenium levels may, however, not be a valid measure during acute phase response to infections, as it may decline in response to an acute infection. The most common functional index of selenium status is glutathione peroxidase activity in the plasma, erythrocytes or whole blood (Friis et al., 2002, p. 191 - 193).

In several cross-sectional studies blood selenium and glutathione peroxidase activity were lower in HIV-infected individuals than non-infected individuals and protective associations were noted between plasma selenium levels and mortality. No published data are available regarding any randomised clinical trials (Fawzi, 2003; Friis et al., 2002, p. 194; Dworkin, 1994). In a small observational study on HIV-infected children (n=24), it was demonstrated that low levels of plasma selenium were significantly and independently related to mortality. Children had a significant six-fold higher risk of mortality with low selenium levels (Campa et al., 1999), while children with low glutathione concentrations had a higher viral load (Rodriguez et al., 1998). This was recently confirmed in a study by Kupka et al. (2005) when it was demonstrated that

plasma selenium levels among children born to HIV-infected mothers, were inversely associated with risk of all cause mortality.

ii) Vitamin A: The only micronutrient properly researched in HIV-infected children

Vitamin A or trans-retinol plays a vital role in immunity (growth and function of T and B cells), reproduction, maintenance of epithelial surfaces, growth and vision. Vitamin A has long been recognised to impact on the morbidity and mortality of infectious diseases (Semba, 2002, p. 74). Vitamin A deficiency is rare in **developed** countries with infants, children and pregnant women being the highest risk groups (Semba et al., 1995). In **developing** countries, vitamin A deficiency is notable in up to 80 percent of stable HIV-infected children (Periquet et al., 1995).

Vitamin A is by far the most investigated micronutrient in children with HIV/AIDS. Children born to HIV-infected, vitamin A deficient mothers are much more likely to experience growth failure, than children born to mothers with normal vitamin A levels (Semba et al., 1997). Although some HIV-infected children seem to have normal vitamin A levels, deficiencies have been reported on numerous occasions (Periquet et al., 1995). Low blood levels are associated with faster disease progression and increased mortality in HIV-infected adults (Baum et al. 1995; Semba et al. 1995).

In a study on HIV-infected adults in Cape Town (Visser et al. 2003), a weak positive association between $CD4^+$ cell count, as well as the CD4:CD8 ratio and plasma retinol levels were demonstrated. In these patients a threefold increased risk of low plasma retinol were demonstrated with advanced disease (WHO – stage 4). Higher body weight in adults had a significantly protective effect in terms of development of vitamin A

deficiency. In another study (Semba et al., 1994), low plasma levels of vitamin A have been associated with a significant higher risk of death. Read et al. (1999) demonstrated that in HIV-infected children in developed countries with normal vitamin A levels, the latter did not appear to be associated with morbidity or mortality.

Most intervention studies indicate that vitamin A supplementation in adults with HIV may only be important in reversing the deficiency (Ramakrishnan et al., 2004, p. 104), as improvements in relation to viral loads and $CD4^+$ cell counts could not be established (Fawzi et al., 1998).

Supplementation of 181 HIV-infected children with 60 mg retinol equivalent, indicated that vitamin A supplementation decreased mortality and children had a lower prevalence of persistent cough, chronic diarrhoea and a shorter duration of ear discharge. There was no significant impact on prevalence of fever, bloody stools or hospitalisations in the vitamin A supplemented group (Semba et al., 2005). In other studies, high dose vitamin A supplementation has shown to reduce diarrhoeal disease morbidity and AIDS-related mortality in HIV-infected children as well as improve $CD4^+$ cell counts of children with AIDS (Fawzi et al., 1999; Coutsoudis et al. 1995). In infants with severe diarrhoea (unknown HIV status, but HIV endemic area), vitamin A supplementation improved vitamin A stores, although significant clinical benefits could not be established (Rollins et al., 2000). Apart from one concern where vitamin A supplementation was associated with higher mortality in breast-fed infants who were polymerase chain reaction (PCR) negative at birth (Humphrey et al, 2006), universal vitamin A supplementation in HIV-endemic areas seem significantly to impact on infant morbidity and mortality.

iii) Vitamin D

The active form of vitamin D, 1,25 – dihydroxyvitamin D_3, has been shown to act as a powerful immunoregulator (Ramakrishnan et al., 2004, p.107). Levels of the active form of vitamin D were found to be severely deficient among HIV-infected subjects (53% had values below normal range and 33% had undetectable levels), with the lowest levels in those with the most clinically advanced disease (Haug et al., 1994). No studies have been done on HIV-infected children to determine significance.

iv) Vitamin E

Vitamin E is the collective name for all the compounds known as tocopherols and tocotrienols of which ά-tocopherol has the highest biological activity. Vitamin E is a major antioxidant with the main function of acting as a free radical scavenger to protect lipid membranes against oxidative damage. It is also involved in the regulation of the immune response (Tang & Smit, 2002, p. 118).

Total serum or plasma tocopherol levels are often used to assess vitamin E status. In HIV-infected adults it was demonstrated that high serum levels of vitamin E (> 23.5 micromol/l) may be associated with a significant decrease in the risk of HIV-1 disease progression (Tang et al, 1997). However, low serum levels of vitamin E were not associated with faster progression. Other studies (Pacht et al., 1997) suggested that decreasing levels of vitamin E over time were associated with significant declines in $CD4^+$ cell counts and increased progression to AIDS.

In two studies (Mastroiacovo et al., 1996; Periquet et al., 1995) on 21 and 20 HIV-infected children respectively, it was reported that the HIV-infected children had significantly lower vitamin E values than HIV-negative controls. Mean vitamin E levels were significantly lower in the infected group, despite supplementation with multivitamins which included 1.5 mg vitamin E (Mastroiacovo et al., 1996). In contrast, other researchers (Omene et al., 1996) found no difference in vitamin E levels between HIV-infected (n=12) and HIV-negative (n=14) children.

v) Zinc

Zinc is of particular importance in the regulation of the immune system because of its role in apoptosis and oxidative stress. Zinc deficiency may lead to an increased rate of apoptosis in thymic lymphocytes and other cells. It also plays a role in protection against oxidative stress through the antioxidant Zn,Cu superoxide dismutase (Friis & Sandstrom, 2002, p. 160). Several factors appear to contribute to low plasma zinc concentration or zinc deficiency in HIV-infection. Dietary insufficiency, poor appetite, reduced total food intake and frequent nausea and vomiting are likely to increase the risk of zinc deficiency. Increased zinc losses are also recorded in children with diarrhoeal disease (Castillo-Duran et al., 1998). To aggravate the situation, zinc deficiency seems to be associated with poor gut lining integrity, which interferes with macro- and micronutrient absorption. Increased plasma tumor necrosis factor levels as well as inflammatory mucosal reactions may compromise absorption, while the overproduction of cytokines may increase renal zinc wasting (Siberry et al., 2002). Apart from the impact of zinc on the immune system, deficiency is also associated with slower growth in children (Coovadia & Bobat, 2002).

Determination of zinc deficiency is limited by a number of factors. While plasma zinc levels are easily measured, it may not reflect true body zinc status. Plasma zinc decreases in the presence of an acute phase response, even in the absence of a true zinc deficiency (Keen et al., 2004, p. 132). This may be particularly confounding in the setting of HIV infection where concurrent illnesses are common. In a retrospective study of hospitalised HIV-infected adults, the incidence of bacterial infections was inversely correlated in dose-response fashion with serum zinc concentration during the first week of hospitalization (Koch et al., 1996). Despite the limitations, most studies evaluating zinc status have measured plasma or serum levels and varying rates of low plasma levels have been detected (Siberry et al., 2002). Low zinc levels were found to be highly prevalent in HIV-infected adults (Baum et al., 1997), especially if the disease state deteriorates (Bogden et al., 2000). One of the few studies to evaluate zinc status in children (n=24) did not find the children to be zinc deficient (Henderson et al. 1997), with none of the children having zinc deficiency despite a low mean intake in the group.

In some studies amongst adults, a more advanced disease stage and lower $CD4^+$ cell counts have been associated with lower zinc concentrations – it is, however, not clear if the low plasma zinc concentrations are a consequence, confounder or contributing cause of more severe HIV-related disease (Siberry et al., 2002).

In earlier studies before ARVs were freely available, low serum zinc concentrations acted as a marker of disease activity instead of reflecting true zinc status (Graham et al., 1991). In a later report, Baum et al. (1997) found that low plasma zinc individually predicted mortality in HIV-infected patients, controlling for $CD4^+$ cell counts. Campa et al. (1999) as well as Periquet et al. (1995) could not establish a similar prediction in 24 and 21 symptomatic HIV-infected children respectively. However, several studies have

suggested that zinc supplementation decreases paediatric morbidity and mortality – in particular due to respiratory and diarrhoeal diseases (Bobat et al., 2006; Siberry et al., 2002), without a risk of increasing the viral load.

Although zinc is essential for proper immune function as well as integrity of mucosal surfaces, increased zinc intake was shown to be associated with potentially adverse effects on HIV disease progression in early observational studies (Tang et al., 1996). Zinc binds to viral nucleocapsid protein Ncp7 and forms zinc fingers, which are essential in pro-viral DNA synthesis and the activity of reverse transcriptase – thus the concern that zinc supplementation may promote, rather than inhibit, viral replication (Tanchou et al., 1998). Zn supplementation of 10 mg per day did not lead to an increase in plasma HIV-1 viral load and the children were less likely to develop watery diarrhoea than the HIV-infected children in the placebo arm (Bobat et al., 2006). Dietary zinc intake correlated with the plasma zinc concentration in most asymptomatic HIV-infected subjects.

2.5.2.2 Other micronutrients and disease progression

i) Folate

Folate act as a coenzyme in DNA synthesis and amino acid metabolism and a deficiency is normally characterised by a megaloblastic anaemia. Folate does not play any role in maintenance of the immune system. Although folate deficiency has been reported in HIV-infected adults – especially those with a low folate intake, folate deficiency is not associated with faster disease progression (Tang et al., 1997). In one of the only studies to evaluate folate deficiency in HIV-infected children (n=60), no children presented with

deficient levels, while 55 percent had values above the normal range for age (Eley et al., 2002b). In this study there was no relationship between plasma folate and CRP concentrations – therefore folate is probably not involved in the acute phase response in HIV infection.

ii) Vitamin B 12

The function of vitamin B12 is closely related to the function of folate. Vitamin B12 has a coenzyme function that catalyses the metylation of homocystein to form methione. The latter assists in the regeneration of unmethylated folate which is critical in the synthesis of nucleic acid. Vitamin B 12 deficiency is mostly the result of inadequate absorption and not necessarily insufficient dietary intake. Vitamin B12 deficiency is associated with neurological disorders and macrocytic, megaloblastic anaemia (Garland & Fawzi, 2002, p. 102). Vitamin B12 seems to enhance T-cell proliferation. In a cross-sectional study on HIV-negative adults, 14 subjects with vitamin B12 deficiency anaemia had significantly lower numbers of lymphocytes and $CD8^+$ cells, which were reversed after vitamin B12 injections (Tamura et al., 1999).

Vitamin B12 levels have been shown to decrease with disease progression (Rule et al., 1994) and the prevalence of deficiency is especially high in patients with chronic diarrhoea (Ehrenpreis et al., 1994). Studies to look at the relationship between vitamin B12 and neurological symptoms associated with HIV disease reported inconsistent results. In small uncontrolled studies, vitamin B12 injections were associated with improvement of neurological disorders and possibly an improvement in cognitive function (Garland & Fawzi, 2002, p.107). According to Eley et al. (2002b) five percent of HIV-infected children (n=60) presented with deficient vitamin B12 levels, while 10

percent presented with values above normal range for age. Concentrations of vitamin B12 were significantly lower in severely immunosuppressed children.

2.5.3 Health status, wasting and disease progression

There are no data on the relationships of other micronutrients and child health among HIV-infected children (Fawzi, 2003). Available data from cohort studies in developed countries indicate that the median survival of children with vertically acquired HIV infection from the time of diagnosis is in excess of five years, while in Africa, it is significantly shorter. Data from the USA a number of years ago (Barnhart et al., 1996), indicated that an HIV-infected child had a 75 percent chance of survival until the age of five years. In Uganda the median survival was only 21 months, with South Africa only slightly better off with a median survival of 32 months (Hussey et al., 1998). Of the 193 children monitored in the latter study, 24 percent fell into category A, 58 percent into category B and 18 percent into category C of the CDC clinical classification. Category C was significantly associated with a higher risk of death, especially in younger children.

The clinical findings that can be found on assessment of HIV-infected children with **rapid progression of disease** are summarised in Table 2.7 as described by Nielsen (1999). These clinical findings go hand in hand with the laboratory findings summarised in Table 2.8 (Brittain, 2000; Nielsen, 1999).

Children who exhibit intermediate to slow progression of HIV disease may have an unremarkable clinical history or occasionally a slightly increased incidence of bacterial infections. Additionally, their physical examination may be unremarkable except for occasional lymphadenopathy. Other clinical findings that may be present even in

children with very slow disease progression are: hepatosplenomegaly, increased parotid or tonsillar size, bruising, lymphoid interstitial pneumonitis (LIP) and HIV dermatitis.

Table 2.7: Clinical findings on assessment of HIV-infected children

History of clinical finding	Clinical finding on physical examination
Progressive respiratory distress during first six months of age (PCP) Failure to thrive Developmental delay or loss of milestones Chronic diarrhoea Recurrent bacterial infections (sinusitis, otitis media, pneumonia, meningitis) Recurrent vaginal candidiasis (older girls) Bruising or epistaxis Rash or HIV dermatitis HIV cardiomyopathy Recurrent varicella or herpes zoster Recurrent oral candidiasis Recurrent or disseminated herpes simplex virus (HSV) infection *Candida* dermatitis HIV nephropathy CMV retinopathy Mycobacterial infections (*Mycobacterium avium* complex or tuberculosis)	Generalised lymphadenopathy No lymphadenopathy with very advanced disease Hepatomegaly, splenomegaly or both Increased parotid glands Increased tonsillar size Bruising or petechiae Failure to achieve developmental milestones Hyperreflexia, hyperspasticity, rigidity, increased muscle tone Oral thrush Rash: erythematous papular rash (HIV dermatitis), herpes zoster, eczema, *Candida* dermatitis, herpes simplex Presence of concurrent infections Aphthous ulcers Findings of congestive heart failure Findings of renal failure or nephrotic syndrome Chronic lung disease or lymphoid interstitial pneumonitis Digital clubbing

Table 2.8: Laboratory findings in HIV-infected children

- Anaemia (mostly anaemia of chronic disease)
- Neutropenia
- Thrombocytopenia (common feature of advanced AIDS)
- Hypergammaglobulinaemia (frequent)
- Hypogammaglobulinaemia (in advanced disease)
- Failure to form antibodies following vaccination
- Anergy on skin testing
- Decreased absolute $CD4^+$ counts for age:

< 1 yr: < 1750 cells/mm^3
1- 2 yr: < 1000 cells/mm^3
2- 6 yr: < 750 cells/mm^3
< 6 yr: < 500 cells/mm^3

- Decreased absolute CD4 percentages for age:

< 1 yr: < 30%
1- 2 yr: < 25%
2- 6 yr: < 20%
< 6 yr: < 20%

- Inverted $CD4^+$/ $CD8^+$ ratio
- Increased liver transaminases
- Increased amylase (due to parotitis)
- Increased lactic dehydrogenase levels
- Abnormal chest X ray
- Brain atrophy on magnetic resonance image or computed tomographic scan
- Positive urine cultures for CMV

2.5.4 Other metabolic parameters indicative of health status

2.5.4.1 Serum albumin level as indicator of immune function

A low serum albumin level may in certain conditions be an indicator of compromised nutritional status. According to the CDC (1994) a serum albumin of less than 30g/l is part of the definition of wasting syndrome and one of the major AIDS defining illnesses. In a study by Guenter et al. (1993) on 77 HIV-infected adults, it was indicated that the risk of

death was 3.6 times higher for patients with serum albumin levels below 3.5 g/dl, even after controlling for age and $CD4^+$ count. The value of serum albumin levels as prognostic indicator was supported by other investigators (Villamor et al., 2005; Feldman et al., 2000). In a group of 298 HIV-infected children, children who were classified as survivors had a significantly higher serum albumin level than the non-survivors (Shearer et al., 2000). This is supported by research from Heller et al. (2000) where it was demonstrated that serum albumin levels were strongly reliable as an instrument to evaluate and monitor nutritional risk in a group of 39 HIV-infected children. In the latter study, serum albumin was more reliable than the more sensitive markers, namely pre-albumin or transferrin. Outcomes from a study by Eley et al. (2002a) supported the research above, as HIV-infected children with category 3 disease, presented with a lower median albumin concentration than children with category 1 disease.

2.5.4.2 Serum cholesterol

Melvin (2001) reported a mean serum cholesterol value for HIV-infected children not on ARVs of 3.69 mmol/l. Total cholesterol levels of HIV-infected children not on protease inhibitors (PIs), are significantly lower than those receiving PIs.

2.5.4.3 Haemoglobin

Anaemia is a common manifestation of paediatric HIV infection with anaemia of chronic disease the most frequent type (Brittain, 2000). In a group of 60 antiretroviral naïve HIV-infected children in Cape Town, 73 percent presented with low haemoglobin (Hb) levels (Eley et al., 2002b). There was also a significant relationship between immunological and disease status and the median Hb levels found. Several other studies (Villamor et

al., 2005; Shearer et al., 2000) indicated that Hb levels were higher in children who survived longer and that anaemia could be used as an indicator to predict mortality. According to the WHO (2006) children with an Hb below 8 g/dl should be classified as Clinical Stage 3.

2.5.4.4 Ferritin

Iron deficiency is the most common mineral deficiency in the world and most prevalent in young children. Although plasma ferritin is a measure of iron stores, it is an acute phase protein that may be elevated in inflammation (Keen et al., 2004, p.125). A recent study however indicated that a low prevalence of inflammation has little influence on the distribution of ferritin and that levels of biomarkers for acute phase response do not necessarily predict elevated ferritin levels (Beard, 2006). Serum levels of ferritin should therefore be interpreted with caution.

2.6 Management of HIV-infected children

The ultimate goal of health professionals caring for infants and children with HIV/AIDS is to improve morbidity, mortality and quality of life (Garg & Miller, 1999, p. 117). Infants and children diagnosed with HIV-infection should be followed up regularly. Accepted standards of care should include the following (WHO, 2006; Rabie et al., 2006):

- Initiation of co-trimoxazole *Pneumocystis jerovici* (PJP or PCP) prophylaxis;
- Regular clinical assessment for HAART eligibility as well as management of secondary infections;

- Constant monitoring for documented tuberculosis exposure or symptoms suggestive of the disease;
- Basic child health promotion, including routine vaccinations, deworming and nutritional supplementation;
- Optimal care and support of the mother and/or caretaker.

2.6.1 Nutrition support in HIV-infected children

A proactive approach to nutrition support in HIV-infected children is recommended by the WHO (2006) because of the increased nutritional needs associated with infection and growth. Nutrition support should include an adequate nutrient intake based on locally available and affordable foods and a daily intake of micronutrients equivalent to one RDA.

2.6.2 Goals of nutrition support

Other than in adults, relatively small changes in growth, fat and muscle stores in children should receive close attention. Early intervention is far more effective than attempts at repletion, once the nutritional status has been compromised (WHO, 2006). Early intervention is only possible if children receive regular nutritional assessments which include anthropometry, laboratory measurements and clinical indicators. Information on dietary intake, feeding skills, caregivers, food availability, use of nutritional supplements and herbal or complementary remedies should also be included in the assessment (Heller, 2000b).

The goals of nutrition support in HIV-infected children include:

- Prevention of protein-energy malnutrition and/or growth failure;
- Prevention of micronutrient deficiencies;
- Management of disorders of the gastrointestinal tract (Steenkamp & Dannhauser, 2001).

2.6.3 Recommendations for macro and micronutrient supplementation in HIV-infected children

The energy intake of asymptomatic children should be increased by 10 percent and by 20 to 30 percent if they are symptomatic or recovering from acute illness. Energy needs should be further increased if nutritional deficiencies exist. The protein content of the diet should be in accordance with the recommendation of 12 to 15 percent of total energy intake, as recommended for a normal balanced diet (WHO, 2003).

In children with growth failure, despite adequate oral intake, or those with feeding difficulties, more targeted support may be necessary. High-energy foods or supplements should be considered for children with conditions that interfere with normal intake or digestion (WHO, 2006; Hanna, 1996). For children in developed countries, who failed to demonstrate adequate response to measures described above, appetite stimulants (megestrol acetate), total enteral nutrition by means of gastrostomy tube or total parenteral alimentation (TPN) have proved successfully to reverse symptoms of malnutrition. Although these nutrition interventions improved weight gain in HIV-infected children, the majority of the children could not be weaned of the tube feeds or TPN

(Heller, 2000; Rothpletz-Puglia et al., 1998). The viability of these nutrition interventions in developing countries should, however, be questioned and has never been tested.

2.6.3.1 Outcome of nutrition intervention trials

In developed countries energy dense nutrition supplements often form part of the nutrition intervention strategy; however, few studies have evaluated the impact of nutrition supplementation (Visser, 2005, p. 467). Two separate intervention studies have shown that gastrostomy tube feeding results in increased body weight in 18 (Henderson et al., 1994) and 23 children (Miller et al., 1995) with HIV, respectively. Although enteral nutrition was not associated with increases in $CD4^+$ cell counts, children with higher initial $CD4^+$ counts responded better to clinical nutrition support in terms of weight gain.

In a study by Guarino et al. (2002), it was shown that nutrition intervention in the form of TPN (n=46) or enteral nutrition (EN) (n=16) improved the bodyweight in both groups by at least five percent from baseline. A parallel improvement was demonstrated in nutritional status and absorptive intestinal function, which indicates that malabsorption in HIV infection may be directly related to malnutrition. The data strongly suggest that nutritional interventions that are early, affordable and available may have a dramatic impact on the course of HIV disease in children.

An increase in total lymphocyte numbers has been observed in HIV-infected adults receiving enteral alimentation, although $CD4^+$ cell counts did not change. The hypothesis was posed that this failure could be caused by a direct viral cytopathic effect. Unlike in adults, naïve $CD4^+$ cell counts increased in children after HAART, and this relates to thymus function. Therefore nutritional rehabilitation may increase naïve $CD4^+$ cell

counts, thereby allowing partial functional recovery of the immune response in children with HIV-infection.

2.6.4 Nutrition supplementation strategies in resource poor settings

According to Morgan et al. (1997), progression rates of AIDS in Africa are similar to those in developed countries; however, rates of all-cause mortality are much higher and progression times to death are shorter than in developed countries. Nutritional care and support may therefore be of more benefit to people living with HIV/AIDS in developing countries, because underlying factors contribute to a poor baseline nutritional status, which impacts on disease progression and outcome (Visser, 2005, p. 467). Targeted feeding programmes may face many challenges including identification of HIV infected individuals as well as stigma-related issues (Thorne-Lyman et.al., 2004).

In general, child nutrition can be improved by increasing the intake of nutrients or to reduce the occurrence of disease. The goals of nutrition support should be to increase the amount and frequency of food intake. The amounts can be increased by giving larger servings and to encourage the child to eat the whole serving. To increase the energy and nutrient density of foods, protein and micronutrients should be added or amylase can be added to reduce the viscosity (thickness) of solid foods. Ascorbic acid should be added if phytate is present in the raw material, but only after the product has been pre-cooked (Lutter & Dewey, 2003; Gopaldas & Chinnama, 1992). According to Hussey et al. (2005), in situations where micronutrient deficiencies are endemic like in most developing countries, these nutrients should be provided through food fortification or micronutrient supplements, which should provide at least one to two times the RDA. The most common form of supplemental feeds used in developing countries to increase

energy and nutrient intake, includes blended maize/soy flour, food parcels or ready-to-use food supplements (RTUF). Although a number of developing countries have implemented programmes to supplement diets of people infected with HIV (including children) through community and home-based efforts, there are no recent reports that provide detail on staging, improvements in clinical status or quantitative evaluations of the impact of the interventions. It is therefore difficult to draw any conclusions as to the efficacy of these interventions on disease progression.

2.6.4.1 Ready-to-use food (RTUF)

In RTUF, powdered ingredients are embedded in a lipid rich paste, which results in an energy dense product that resists microbial contamination. A study by Manary et al. (2004) has proved that 95 percent of children (unknown HIV status) receiving RTUF in quantities sufficient to meet requirements for full catch-up growth, managed to reach graduation weight, as opposed to 78 percent of children receiving supplemental amounts of RTUF or a maize/soy blend. The children on the maize/soy blend, however, experienced significantly less diarrhoea than those on the RTUF. Overall results with all three of the supplements indicate a recovery rate of 84 percent, with children on RTUF experiencing a growth rate of 5.1 g/kg/day, which is less than projected growth rates of 7 to 15 g/kg/day which have been reported from well functioning inpatient feeding centres (Khanum et al., 1994). Children on the RTUF supplement as well as the maize/soy blend had a growth rate of less than the 5.1 g/kg/day, which suggests that children need an energy-dense diet and not an energy-dense snack in order to obtain catch-up growth (Manary et al. 2004).

2.6.4.2 Maize-soy blends: rationale behind enzyme modification and micronutrient enrichment

Most people in developing countries subsist on staple foods like wheat, maize, rice, cassava or beans, which are all inexpensive sources of energy, but poor sources of other nutrients. Modification of these staple foods to increase either micronutrient content or bioavailibilty can be very important (Friis et al., 2002, p. 232).

Instant fortified soy/maize supplements have been in use for a number of years in most of the provinces in South Africa as part of the health facility based programme, previously called the protein-energy-malnutrition scheme (Department of Health, 2004). A large number of African countries were making use of supplementary foods in the past as part of feeding schemes, with varying degrees of success (Beaton & Ghassemi, 1982). Some of these supplemental foods have come under scrutiny due to low bio-availability of iron and zinc, due to high concentrations of phytic acid. According to Gibson et al. (1998) research on the feasibility of fortifying plant-based complementary foods with protected fortificants of calcium, iron and zinc, which do not bind with phytic acid, was urgently required. Another option would be partly to remove the water soluble phytates by either soaking or by the hydrolysis of the phytates, thereby increasing the absorption of zinc and iron. By further fortifying the staple with other micronutrients, most of the intrinsic deficiencies can be overcome (Friis et al., 2002, p. 232).

Dietary bulk is a major limiting factor in infant feeding, especially during rehabilitation of malnutrition. Although optimal nutrition management of infants and young children should include guidelines on frequent feedings, the latter seems to be a problem in low income groups as mothers feed children less than three times per day. Children between

the ages of one and 10 years, under normal conditions, need between 55 and 100 kcal/kg/day (230 and 420 kJ/kg/day), decreasing over time as they get older (Escott-Stump, 2002, p.20). HIV-infected children may need double that amount to support growth and development. In order to consume these high energy diets, targeted food supplementation with energy dense fortified maize-soy blends have been used in many Africa countries for a number of years. These extruded cereal-pulse mixes can be improved by the addition of 5 percent commercial barley malt instead of sugar (Gopaldas, 1998).

From preliminary studies in Peru and Guatemala (Brown, 1991), it is known that the functional gastric capacity of young children is approximately 40 to 50 g/kg bodyweight per feeding. In practice, it relates to 300 to 500 g of reconstituted food supplement for a child between six kg and 10 kg. Although most children from the age of 12 months and older will therefore be able to consume the recommended portion of 100g dry product (300 g wet product), the presence of infection may cause loss of appetite and delayed gastric emptying which can lead to a decrease in food and energy intake. The total solids content of a diet based on starch-containing foods may be limited by the resulting thickness or viscosity of the cooked product. The viscosity of such a mix can be reduced without sacrificing its energy density by adding an exogenous source of amylase to liquefy the gelatinised starch. A number of studies (Den Besten et al., 1998; Chinnama & Gopaldas, 1993) proved that the addition of amylase to a starch based diet can significantly increase energy intake in children.

Several strategies can be used to enhance mineral bio-availability in cereal-based complementary foods, by reducing the content of phytic acid. All these methods induce enzymatic or non-enzymatic hydrolysis of phytic acid (hexa-inositol phosphate) and

penta-inositol phosphate that do not inhibit zinc or iron absorption. The bio-availability of iron and zinc can also be improved by promoting the intake of enhancers; for example, ascorbic acid (Gibson et al, 1998).

Research by Gopaldas and Chinnama (1992) shown that infants consume significantly greater amounts of fully malted mixes of staples than the equivalent roasted mix. Catalytic amounts of any germinated cereal grain can liquefy virtually any cereal-based viscous gruel, due to the presence of alpha-amylase, which breaks down the starch molecules into smaller units at boiling temperatures. Better results were achieved with regard to energy intake and growth if diets were supplemented with these alpha-amylase fortified cereals, instead of the locally available products. In a local study on 30 healthy children (Den Besten et al., 1998), subjects consuming a diet fortified with alpha-amylase, experienced a 23 percent increase in energy intake and a 10 percent increase in protein intake. The benefits seemed to be most pronounced if the breakfast was replaced. Even though these subjects consumed less food during the rest of the day, the benefit from the increased intake outweighed the fact that they ate less later on.

Findings as summarised by Long and Santos (1999) indicated that micronutrient supplementation can be used as an important tool to reduce morbidity and mortality in children in developing countries. Although food-based approaches will always be the intervention of choice, HIV-infected individuals have macro and micronutrient needs that far exceed those of HIV-negative individuals, and supplements may make it easier to reach those goals (Friis et al., 2002, p. 233).

According to the WHO (2003), micronutrients have been proposed as a low-cost immune modulating intervention that may slow progression of HIV to AIDS. If onset of

advanced disease can be delayed, the start of ARVs can be delayed as well, reducing drug-related adverse effects and costs.

2.7 Nutrition intervention and the impact on immune function in children

Aggressive nutritional supplementation by means of gastrostomy tube feedings in a sample of 23 HIV-infected children showed no improvement in $CD4^+$ cell counts after a six month intervention period, despite significant improvements in the weight-for-age Z-scores and better outcome (Miller et al., 1995). In order to facilitate improvements in weight, the median energy intake was increased from 110kcal/kg/day (462 kJ/kg/day) before enteral supplementation to 189kcal/kg/day (794 kJ/kg/day) during gastrostomy tube feedings.

In a study by Guarino et al. (2002), it was shown that nutrition intervention in the form of TPN (n=46) or enteral nutrition (n=16) may restore intestinal absorption and increase $CD4^+$ cell numbers. The duration of the intervention was 142 days for EN and 157 days for TPN. All received reverse transcriptase inhibitors prior to the intervention. Children in the EN group had a baseline weight for age Z score of -1.5 and -2 in the TPN group. The majority of the children in both groups experienced an increase in bodyweight of at least 5 percent from baseline. The EN group experienced a significant increase in $CD4^+$ cell counts, while the TPN group's values did not improve.

2.8 Nutrition intervention and the health status of children

According to Gerntholz and Richter (2004) it is important to note that legal barriers exist, making it difficult for certain categories of HIV infected children, to access ARVs. One

such group includes children without parents or legal guardians. Unfortunately some sources already indicate that as much as 13% of children below the age of 15 years might have lost one or more parents as a result from the disease (Shisana & Shisana, 2002). In those children where administration of ARVs might be delayed, nutrition support plays a vital role in management of the infection.

2.9 Summary

Nutritional problems are mostly chronic and nearly universal in HIV-infected children and data strongly suggest that growth and micronutrient deficiencies impact on immune parameters and outcome. Health professionals need to be aware of children's nutritional status and endeavor to preserve proper growth. Despite several programmes being run by government, non governmental organisations and faith based organisations, no peer reviewed publications are available on the impact of these interventions.

Micronutrient supplementation can be used as an important tool to reduce morbidity and mortality in children in developing countries. Micronutrients are included at RDA levels in a number of enriched soy/maize blends used to supplement the diets of children living with HIV/AIDS.

The viscosity of such a mix can be reduced without sacrificing its energy density by adding an exogenous source of amylase to liquefy the gelatinised starch. Several studies proved that the addition of amylase to a starch based diet can significantly increase energy intake in children. Enzymatic modification may also play a role in hydrolysis of phytic acid, thereby enhancing mineral bio-availability in cereal-based complementary foods.

Several data sources suggest that nutrition interventions that are early, affordable and largely available may have a dramatic impact on the course of HIV infection in children.

2.10 References

Allard, JP. 2002. Oxidative stress and Infections, in Micronutrients in HIV Infection. Ed. by H. Friis. London: CRC Press, pp. 43 – 72.

Allard, J.P., Aghdassi, E., Chau, J., Tam, C., Kovacs, C.M., Salit, I.E. and Walmsley, S.L. 1998. Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. 1998. Acquired Immune Deficiency Syndromes, 12(13): 1653 – 1659.

Allen, D.M., Simelela, N.P. and Makubalo, L. 2000. Epidemiology of HIV/AIDS in South Africa. The South African Journal of HIV Medicine, 1: 9 – 11.

Arpadi, S.M. 2005. Growth failure in HIV-infected children. Consultation on Nutrition and HIV/AIDS in Africa: Evidence, lessons and recommendations for action, World Health Organisation, Durban.

Arpadi, S.M., Cuff, P.A., Kotler, D.P., Wang, J., Bamji, M., Lange, M., Pierson, R.N. and Matthews, D.E. 2000. Growth velocity, fat-free mass and energy intake are inversely related to viral load in HIV-infected children. Journal of Nutrition, 130: 2498 – 2502.

Barnhart, H.X., Caldwell, M.B., Thomas, P., Mascola, L., Ortiz, I., Hsu, H.W. 1996. Natural history of human immunodeficiency virus disease in perinatally infected children: an analysis from the pediatric spectrum of disease project. Pediatrics, 97: 710 – 716.

Bateman, C. 2004. AIDS Pandemic – Still time to prepare for the orphan tide. South African Medical Journal, 94(7): 505 – 507.

Baum, M.K., Shor-Posner, G., Lu, Y., Rosner, B., Sauberlich, H.E., Fletcher, M.A., Szapocznik, J., Eisdorfer, C., Buring, J.E. and Hennekens, C.H. 1995. Micronutrients and HIV-1 disease progression. Acquired Immuno Deficiency Syndromes, 9(9): 1051 – 1056.

Baum, M.K., Shor-Posner, G., Lai, S., Zhang, G., Lai, H., Fletcher, M.A., Sauberlich, H. and Page, J.B. 1997. High Risk of HIV-Related Mortality is Associated with Selenium Deficiency. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology, 15: 370 – 374.

Beard, J.L., Murray-Kolb, L.E., Rosales, F.J., Solomons, N.W. and Angelilli, M.U. 2006. Interpretation of serum ferritin concentrations as indicators of total-body iron stores in survey populations: the role of biomarkers for the acute phase response. American Journal of Clinical Nutrition, 84: 1498 – 1505.

Beaton, G.H. and Ghassemi, H. 1982. Supplementary feeding programs for young children in developing countries. American Journal of Clinical Nutrition, 35(9): 864 – 916.

Beisel, W.R. 1996. Nutrition and Immune Function: Overview. Journal of Nutrition, 126: 2611S – 2615S.

Benjamin, D.K., Miller, W.C., Benjamin, D.K., Ryder, R.W., Weber, D.J., Walter, E. and McKinney, R.E. 2003. A comparison of height and weight velocity as part of the composite end point in pediatric HIV. Acquired Immuno Deficiency Syndromes, 17(6): 2331 – 2336.

Berhane, R., Bagenda, D., Marum, L., Aceng, E., Ndugwa, C., Bosch, R.J. and Olness, K. 1997. Growth failure as a prognostic indicator of mortality in pediatric HIV infection (Abstract). Pediatrics,100(1): E7.

Bobat, R., Coovadia, H., Moodley, D. and Coutsoudis, A. 1999. Mortality in a cohort of children born to HIV-1 infected women from Durban, South Africa. South African Medical Journal, 89(6): 646 – 648.

Bobat, R., Coovadia, H., Stephan, C., Naidoo, K.L., McKerrow, N., Black, R.E. and Moss, W.J. 2006. Safety and efficacy of zinc supplementation for children with HIV-1 infection in South Africa: a randomized double-blind placebo controlled trial. Lancet, 367: 814 – 815.

Bogden, J.D., Kemp, F.W., Han, S. 2000. Status of selected nutrients and progression of human immunodeficiency virus type 1 infection. American Journal of Clinical Nutrition, 72: 809 – 815.

Brittain, D. 2000. The Haematology of HIV Infection. Southern African Journal of HIV Medicine, 37 – 38.

Brown, K.H. 1991. Appropriate Diets for the Rehabilitation of Malnourished Children in the Community Setting. Acta Paediatrics of Scandinavia, 374: 151 – 159.

Campa, A., Shor-Posner, G., Indacochea, F., Zhang, G., Lai, H., Asthana, D., Scott, G.B. and Baum, M.K. 1999. Mortality risk in selenium-deficient HIV-positive children. Journal of Acquired Imummunodeficiency Syndromes and Retrovirology, 20: 508 – 513.

Cant, A.J., Gibb, D.M., Davies, E.G., Cale, C. and Gennery, A.R. 2003. HIV Infection, in Forfar & Arneil's Textbook of Pediatrics. Ed by McIntosh, N., Helms, P. and Smyth, R. 6th ed. Spain: Churchill Livingstone, pp. 1295 – 1309.

Carey, V.J., Yong, F.H., Frenkel, L.M. and McKinney, R.E. Jr. 1998. Pediatric AIDS Prognosis using somatic growth velocity. Acquired Immuno Deficiency Syndromes, 12: 1361 – 1369.

Castillo-Duran, C., Vial, P. and Uauy, R. 1998. Trace mineral balance during acute diarrhoea in infants. Journal of Pediatrics, 113: 452.

Centre for Disease Control and Prevention (CDC). 1994. 1994 Revised Classification System for HIV Infection in Children less than 13 years of age. Official Authorized Agenda: Human Immunodeficiency Virus Infection Codes and Official Guidelines for Coding and Reporting ICD-9-CM. Morbidity and Mortality Weekly Report, 43(RR-12): 2-3.

Chadwick, E.G. and Yogev, R. 1995. Pediatric AIDS. Pediatric Clinics of North America, 42(4): 969 – 989.

Chandra, R.K. 1983. Nutrition, Immunity, and Infection: Present Knowledge and Future Directions. The Lancet : 688 – 691.

Chandra, R.K. 1991. 1990 McCollum Award Lecture. Nutrition and immunity: lessons from the past and new insights into the future. American Journal of Clinical Nutrition, 53: 1087 – 1101.

Chantry, C.J., Byrd, R.S., Englund, J.A., Baker, C.J. and McKinney, R.E. 2003. Growth, survival and viral load in symptomatic childhood human immunodeficiency virus infection. The Pediatric Infectious Disease Journal, 22(12): 1033 – 1039.

Chinnamma, J. and Gopaldas, T. 1993. Evaluation of the impact on growth of a controlled 6-month feeding trial on children fed a complementary feed of a high energy-low bulk gruel versus a high energy - high bulk gruel in addition to their habitual home diet. Journal of Tropical Paediatrics, 39: 16 -22.

Cogill, B. 2003. Anthropometric Indicators Measurement Guide. Food and Nutrition Technical Assistance. 2003 edition, pp. 17 - 38. Available from: http:/www. Fantaproject.org/publications/anthropom.shtml, [Accessed 2004 August 15].

Coombs, R., Weeles, S.L., Hooper, C., Reichelderfer, P.S., D´Aquila, R.T., Japour, A.J., Johnson, V.A., Kuritzkes, D.R., Richman, D.D., Kwok, S., Todd, J., Jackson, J.B.,

DeGruttola, V., Grumpacker, C.S. and Kahn, J. 1996. Association of plasma human immunodeficiency virus type 1 RNA levels with risk of clinical progression in patients with advanced infection. Journal of Infectious Diseases, 174: 704 – 712.

Coovadia, H.M. and Bobat, R. 2002. Zinc deficiency and supplementation in HIV/AIDS. Nutrition Research, 22: 179 – 191.

Cotton, M.F., Schaaf, H.S., Willemsen, E., van Veenendal, M., van Rensburg, A.J. and van Rensburg, E.J. 1998. The burden of mother-to-child transmission of HIV-1 disease in a “low” prevalence region – a five year study of hospitalized children. South African Journal of Epidemiology and Infection, 13: 46 – 49.

Coutsoudis, A., Bobat, R., Coovadia, H., Huhn, L., Tsai, W. and Stein, Z. 1995. The effects of vitamin A supplementation on the morbidity of children born to HIV-infected women. American Journal of Public Health, 85: 1076.

Dabis, F. and Ekpini, E.R. 2002. HIV-1 /AIDS and maternal and child health in Africa. The Lancet, 359: 2097 – 2104.

Delmas-Beauviex, M., Peuchant, E., Couchouron, A., Constans, J., Sergeant, C., Simonoff, M., Pellegrin, J., Leng, B., Conri, C. and Clerc, M. 1996. The enzymatic antioxidant system in blood and glutathione status in human immunodeficiency virus (HIV)-infected patients: effects of supplementation with selenium or β-carotene. American Journal of Clinical Nutrition, 64: 101 – 107.

Den Besten, L., Glatthaar, I.I. and Ijsselmuiden, C.B. 1998. Adding α-amylase to Weaning Food to Increase Dietary Intake in Children. A Randomised Controlled Trial. Journal of Tropical Paediatrics, 44: 4 – 6.

Department of Health. 2004. National HIV and Syphilis Antenatal sero-prevalence survey in South Africa, 2003.

Dworkin, B.M. 1994. Selenium deficiency in HIV infection and the acquired immunodeficiency syndrome (AIDS). Chem Biol Interact, 91: 181 – 186.

Eley, B. and Hussey, G. 1999. Nutrition and human immunodeficiency virus infection in children. South African Medical Journal, 89: 190.

Eley, B., Sive, A., Kossew, G., Abelse, L., Cooper, M. and Hussey, G. 2002a. Growth and micronutrient disturbances in stable, HIV-infected children in Cape Town. Annals of Tropical Paediatrics, 22: 19 – 23.

Eley, B.S., Sive, A.A., Shuttleworth, M. and Hussey, G.D. 2002b. A prospective, cross sectional study of anaemia and peripheral iron status in antiretroviral naive HIV-1 infected children in Cape Town, South Africa. British Medical Journal of Infectious Diseases, 2: 3

Ehrenpreis, E.D., Carlson, S.J., Boorstein, H.L. and Craig, R.M. 1994. Malabsorption and deficiency of vitamin B12 in HIV-infected patients with chronic diarrhoea. Digest Dis Sci, 39: 2159 – 2162.

Escott-Stump, S. 2002. Nutrition and diagnosis-related care, Philadelphia: Lippincott Williams & Wilkens, p.20.

Faber, M., Jogessar, V.B. and Benade, A.J., 2001. Nutritional status and dietary intakes of children aged 2 – 5 years and their caregivers in a rural South African community. International Journal of Food Science and Nutrition, 52(5): 401 – 411.

Fawzi, W.W. and Villamor, E. 2001. Malnutrition and HIV infection. In Nutrition, Immunity, and Infection in Infants and Children. Ed. by Suskind, R.M. and Tontisirin, K., Nestlé Nutrition Workshop Series, Pediatric Program, Vol 45., Philadelphia: Nestec Ltd. Vevey/Lippincott Williams & Wilkins, pp. 371-406.

Fawzi, W., Msamanga, G., Spiegelman, D. and Hunter, D.J. 2005. Studies of Vitamins and Minerals and HIV Transmission and Disease Progression. Journal of Nutrition, 135: 938 – 944.

Fawzi, W.W., Msamanga, G.I., Spiegelman, D., Wei, R., Kapiga, S., Villamor, E., Mwakagile, D., Mugusi, F., Herzmark, E., Essex, M. and Hunter, D.J. 2004. A randomized trial of multivitamin supplements and HIV disease progression and mortality. New England Journal of Medicine, 351: 23 – 32.

Fawzi, W., Mbise, R., Hertzmark, E., Fataki, M., Herrera, M., Ndossi, G. and Spiegelman, D. 1999. A randomized trial of vitamin A supplements in relation to mortality among HIV-infected and uninfected children in Tanzania. The Pediatric Infectious Disease Journal, 18: 127-133.

Fawzi, W. 2003. Micronutrients and HIV-1 Disease Progression among Adults and Children, Working paper for World Health Organisation, May 2003, Geneva.

Feldman, J.G., Burns, D.N., Gange, S.J., Bacchetti, P., Cohen, M., Anastos, K., Nowicki, M., Delapena, R. and Miotti, P. 2000. Serum albumin as a predictor of survival in HIV-infected women in the Women's Interagency HIV Study. Acquired Immuno Deficiency Syndromes, 14: 863 – 870.

Friis, H., Kaestel, P., Iversen, A.N.K. and Bügel, S. 2002. Selenium and HIV Infection, in Micronutrients in HIV Infection, Ed. by Friis, H., London: CRC Press, pp. 183 – 200.

Friis, H. and Michaelsen, K.F. 1998. Micronutrients and HIV infection: A review. European Journal of Clinical Nutrition, 52: 157 – 163.

Friiss, H. 2002. Micronutrients and Infections: An Introduction, in Micronutrients in HIV Infection, Ed. by Friis, H., London: CRC Press, pp. 2 - 21.

Friis, H. and Sandstrom, B. 2002. Zinc and HIV Infection, in Micronutrients in HIV Infection. Ed. by Friis, H., London: CRC Press, pp. 159 – 181.

Friis, H., Gomo, E. and Michaelsen, K. 2002. Micronutrient Interventions and the HIV Pandemic, in Micronutrients in HIV Infection, Ed. by Friis, H., London: CRC Press, pp. 219 – 245.

Gaitan-Cepeda, L., Cashat-Cruz, M., Morales-Aguirre, J.J., Sanchez-Vargas, L., Aquino-Garcia, S., Fragoso-Rios, R., Cuairan-Ruidiaz, V., Avila-Figueroa, C., Morales, J. and

Figueroa, C.A. 2002. Prevalence of oral lesions in Mexican children with perinatally acquired HIV: association with immunologic status, viral load, and gender. AIDS Patient Care STDS [Internet] 16(4): 151 – 156. Available from http://medscape.com/medline/abstract/12015869 [Accessed March 30th, 2006].

Garg, S. and Miller, T.L. 1999. Special considerations for the pediatric patient, in Nutritional aspects of HIV Infection. Ed by Miller, T.L. and Gorbach, S.L., New York: Oxford University Press, pp. 109 - 134.

Garland, M. and Fawzi, W.W., 1999. Antioxidants and progression of human immunofediciency virus (HIV) disease. Nutrition Research, 19(8): 1259 – 1276.

Garland, M. and Fawzi, W.W. 2002. B vitamins and HIV Infection, in Micronutrients in HIV Infection. Ed. by Friss, H., London: CRC Press, pp. 99 – 110.

Gernholz, L. and Richter, M. 2004. Access of abandoned children and orphans with HIV/AIDS to antiretroviral therapy – a legal impasse. South African Medical Journal, 94(11): 910 – 912.

Gibson, R.S., Ferguson, E.L. and Lehrfeld, J. 1998. Complementary foods for infant feeding in developing countries. European Journal of Clinical Nutrition, 52: 764 – 770.

Gisselquist, D., Potterat, J.J. and Brody,S. 2004. HIV transmission during paediatric health care in sub-Saharan Africa - risks and evidence. South African Medical Journal, 94: 109 – 116.

Gopaldas, T. 1998. Fighting infant malnutrition with amylase-rich complementary foods. Nutriview. Issue2: 2 – 4.

Gopaldas, T. and Chinnamma, J. 1992. Evaluation of a controlled 6-month feeding trial on intake by infants and toddlers fed a high energy-low bulk gruel versus a high energy-high bulk gruel in addition to their habitual home diet. Journal of Tropical Paediatrics, 38: 278 - 283.

Gouws, E. and Karim, Q.A. 2005. HIV Infection in South Africa: the evolving epidemic, in HIV/AIDS in South Africa. Ed. by Karim, S.S.A. and Karim, Q.A., Cambridge: Cambridge University Press, pp. 48 – 66.

Gray, C. 2005. Cellular immunity in HIV: A synthesis of responses to preserve self, in HIV/AIDS in South Africa. Ed by Karim, S.S.A. and Karim, Q.A., Cabbridge: Cambridge University Press, pp. 119 – 142.

Graham, N.M., Sorensen, D., Odaka, N., Brookmeyer, R., Chan, D., Willett, W. C., Morris, J. S. and Saah, A. J. 1991. Relationship of serum copper and zinc levels to HIV-1 seropositivity and progression to AIDS. Journal of Acquired Immune Deficiency Syndromes, 4: 976 – 980.

Guarino, A., Spagnuolo, M.I., Giacomet, V., Canani, R.B., Bruzzese, E., Giaquinto, C., Roggero, P., Plebani, A. and Gattinara, G.C. 2002. Effects of Nutritional Rehabilitation on Intestinal Function and on CD4 Cell Number in Children with HIV. Journal of Pediatric Gastroenterology and Nutrition, 34: 366 – 371.

Guenter, P., Muurahainen, N., Simons, G., Kosok, A., Cohan, G.R., Rudenstein, R. and Turner, J.L. 1993. Relationships among nutritional status, disease progression and survival in HIV infection. Journal of Acquired Immune Deficiency Syndromes, 6: 1130 – 1138.

Hanna, L. 1996. HIV/AIDS in children: Overview and Guidelines for Disease Management. Bulletin of Experimental Treatments for AIDS, San Francisco AIDS Foundation, Available from: http://sfaf.org/treatment/beta/b29/b29ped.html. [Accessed October 22, 2006].

Haug, C., Muller, F., Aukrust, P. and Freland, S.S. 1994. Subnormal serum concentration of 1,25-vitamin D in human immunodeficiency virus infection: correlation with degree of immune deficiency and survival. Journal of Infectious Diseases, 69: 889 – 893.

Heller, L. 2000. Nutrition Support for Children with HIV/AIDS, The AIDS Reader, Medscape, 10: 109 – 114.

Heller, L., Fox, S., Hell, K.J. and Church, J.A. 2000. Development of an instrument to assess nutritional risk factors for children infected with HIV. Journal of the American Dietetic Association, 100(3): 323 - 329.

Henderson, R.A., Talusan, K., Hutton, N., Yolken, R.H. and Caballero, B. 1997. Serum and plasma markers of nutritional status in children infected with the human immunodeficiency virus. Journal of the American Dietetic Association, 97: 1377 – 1381.

Henderson, R.A., Saavedra, J.M., Perman, J.A., Hutton, N., Livingston, R.A. and Yolken, R.H. 1994. Effect of enteral tube feeding on growth of children with symptomatic human immunodeficiency virus infection. Journal of Pediatric Gastroenterology Nutrition, 18(4): 429 – 434.

Henderson, R.A., Talusan, K., Hutton, N., Yolken, R.H. and Caballero, B. 1998. Resting energy expenditure and body composition in children with HIV infection. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology, 19: 150 – 157.

Humphrey, J.H., Iliff, P.J., Marinda, E.T., Mutasa, K., Moulton, L.H., Chidawanyika, H., Ward, B.J., Nathoo, K.J., Malaba, L.C., Zijenah, L.S., Zvandasara, P., Ntozini, R., Mzengeza, F., Mahomva, A.I., Ruff, A.J., Mbizvo, M.T., Zungiza, C.D. and ZVITAMBO Group. 2006. Effects of a single large dose of vitamin A, given during the postpartum period to HIV-positive women and their infants, on child HIV-infection, HIV-free survival, and mortality. The Journal of Infectious Diseases, 193: 860 – 871.

Hubley, J. 2002. The AIDS Handbook: A guide to the understanding of AIDS and HIV, Oxford: Macmillan Publishers Limited.

Hussey, G and Eley, B. 1998. Nutritional Management of Measles and HIV infection in children. In Encyclopedia of Human Nutrition. Ed. by Sadler, M., Caballero, B. and Strain, S., London: Academic Press, pp. 1126 - 1132.

Hussey, G., Buys, H., Cowburn, C., Eley, B. and Hendricks, M. 2005. Role of micronutrients in HIV Infection., The South African Journal of HIV Medicine, June issue: 18 – 22.

Hussey, G., Hughes, J., Potgieter, S., Kessow, G., Burgess, J., Keraan, M., Caelse, E. and Beatty, D. 1995. Vitamin A status and supplementation and its effects on immunity in children with Acquired Immune Deficiency Syndrome. Department of Paediatrics, University of Cape Town, South Africa.

Hussey, G.D., Reijnhart, R.M., Sebens, A.M., Burgess, J., Schaaf, S. and Potgieter, S. 1998. Survival of children in Cape Town known to be vertically infected with HIV-1. South African Medical Journal, 88(5): 554 – 558.

Italian Register for HIV Infection in Children. 1994. Features of children perinatally infected with HIV-1 surviving longer than 5 years. The Lancet, 343: 191 – 195.

Johann-Liang, R., O'Neill, L., Cervia, J., Haller, I., Giunta, Y., Licholai, T. and Noel, G.J. 2000. Energy balance, viral burden, insulin-like growth factor-1, interleukin-6 and growth impairment in children infected with human immunodeficiency virus. Acquired Immune Deficiency Syndromes, 14: 683 – 690.

Keen,C.L., Uriu-Adams, J.Y., Ensunsa, J.L. and Gerswin, M.E. 2004. Trace Elements/Minerals and Immunity, in Handbook of Nutrition and Immunity. Ed. by Gershwin, M.E., Nestel, P. and Keen, C.L., New Jersey: Humana Press, New Jersey, pp. 117 – 140.

Khanum, S., Asworth, A. and Huttly, S.R.A. 1994. Controlled trial of three approaches to the treatment of severe malnutrition. The Lancet, 344: 1728 – 1732.

Koch, J., Neal, E.A., Schlott, M.J., Garcia-Shelton, Y.L., Chan, M.F., Weaver, K.E., Cello, J.P. 1996. Zinc levels and infections in hospitalized patients with AIDS. Nutrition, 12: 515 – 518.

Kotler, D.P. 1999. Nutrition and wasting in HIV infection, HIV Clinical Management, 8: Medscape, Inc.

Kotler, D.P., Tierney, A.R., Brenner, S.K., Couture, S., Wang, J., Pierson, R.N. Jr. 1990. Preservation of short-term energy balance in clinically stable patients with AIDS. American Journal of Clinical Nutrition, 51(1): 7 – 15.

Krug, A., Pattinson, R.C. and Power, D.J. 2004. Why children die: An under-5 health care survey in Mafikeng region. South African Medical Journal, 94(3): 202 – 206.

Kupka, R., Msamanga, G.I., Spiegelman, D., Hunter, D.J. and Fawzi, W.W. 2005. Selenium levels in relation to morbidity and mortality among children born to HIV-infected mothers. European Journal of Clinical Nutrition, 59(11): 1250 – 1258.

Lepage, P., Msellati, P., Hitimana, D., Bazubagira, A., Van Goethem, C., Simonon, A., Karita, E., Dequae-Merchadou, L., Van de Perre, P. and Dabis, F. 1996. Growth of human immunodeficiency virus type-1 infected and uninfected children: a prospective cohort study in Kigali, Rwanda. 1988 – 1993. The Pediatric Infectious Disease Journal, 15: 479 – 485.

Long, K.Z. and Santos, J.I. 1999. Vitamins and the regulation of the immune response. The Pediatric Infectious Disease Journal, 1999, vol. 18: 283 – 290.

Lutter, C.K. and Dewey, K.G. 2003. Proposed nutrition composition for fortified complementary foods. Journal of Nutrition, 133(9): 3011S – 3020S.

Macallan, D.C. 1998. Metabolic abnormalities and wasting in human immunodeficiency virus infection. Proceedings of the Nutrition Society, 57: 373 – 380.

Macallan, D.C. 1999. Nutrition and immune function in human immunodeficiency virus infection. Proceedings of the Nutrition Society, 58(3): 743 – 748.

Madhivanan, P., Pothi, S.N., Kumarasamy, N., Yepthomi, T., Venkatesan, L.J.S. and Solomon, S. 2003 Clinical manifestations of HIV infected children. Indian Journal of Pediatrics, 70(8): 615 – 20.

Manary, M.J., Ndkeha, M.J., Ashorn, P., Maleta, K. and Briend, A. 2004. Home based therapy for severe malnutrition with ready-to-use food. Archives of Disease in Childhood, 89: 557 – 561.

Marston, B. and De Kock, K.M. 2004. Multivitamins, nutrition, and antiretroviral therapy for HIV disease in Africa. The New England Journal of Medicine, 351: 78 – 80.

Martin, D. 2000. Appropriate laboratory monitoring of HIV. South African Medical Journal, 90(1): 33 – 35.

Martin, D. 2002. Laboratory monitoring of HIV. Southern African Journal of HIV Medicine, July issue: 38 – 39.

Mastroiacovo, P., Ajassa, C., Berardelli, G., Bruni, R., Catania, N., Fidanza, A., Pace, V. and Zanzoglous, S. 1996. Antioxidant vitamins and immunodeficiency. International Journal for Vitamin and Nutrition Research, 66(2): 141.

McKinney, R.E., Robertson, W.R. and the Duke Pediatric AIDS Clinical Trials Unit. 1993. Effect of human immunodeficiency virus infection on the growth of young children. Journal of Pediatrics, 123: 579 – 582.

Melchior, J.C., Salmon, D., Rigaud, D. Leport, C. Bouvet, E., Detruchis, P., Vilde, J.L., Vachon, F., Coulaud, J.P. and Apfelbaum, M. 1991. Resting energy expenditure is increased in stable malnourished HIV-infected patients. American Journal of Clinical Nutrition, 53: 437 – 441.

Mellors, J.W., Rinaldo, C.R., Gupta, P., White, R.M., Todd, J.A. and Kingsley, L.A. 1996. Prognosis in HIV-infection predicted by the quantity of virus in the plasma. Science, 272: 1167 – 1170.

Melvin, A.J., Lennon, S., Mohan, K.M. and Purnell, J.Q. 2001. Metabolic abnormalities in HIV type-1 infected children treated and not treated with Protease Inhibitors. Journal of Acquired Immune Deficiency Syndrome Research and Human Retroviruses, 17(12): 1117 – 1123.

Miller, T.L., Evans, S.J., Orav, E.J., Morris, V., McIntosh, K. and Winter, H.S. 1993. Growth and body composition in children infected with the human immunodeficiency virus. American Journal of Clinical Nutrition, 57: 588 – 592.

Miller, T.L., Easley, M.S., Zang, W., Orav, J., Bier, D.M., Luder, E., Ting, A., Shearer, W.T., Vargas, J.H. and Lipshultz, S.E. 1991. Maternal and infant factors associated with failure to thrive in children with vertically transmitted human immunodeficiency virus-1 infection: The prospective P^2C^2 human immunodeficiency virus multicentre study. Pediatrics, 108(6): 1287 – 1296.

Miller, T.L., McQuinn, L.B., Orav, E.J. 1997. Endoscopy of the upper gastrointestinal tract as a diagnostic tool for children with human immunodeficiency virus infection. Journal of Pediatrics,130: 766 – 773.

Miller, T.L., Awnetwant, E.L., Evans, S., Morris, V.M., Vasquez, I.M. and McIntyre, K. 1995 Gastrostomy tube supplementation for HIV-infected children. Pediatrics, 4: 696 – 702.

Miller, T.L. 2003. Nutritional aspects of HIV-infected children receiving highly active antiretroviral therapy. Acquired Immune Deficiency Syndrome, 7 (suppl 1): S130 – S140.

Morgan, D. Maude, G.H., Malamba, S.S. 1997. HIV-1 disease progression and AIDS-defining disorders in rural Uganda. The Lancet, 350: 245 – 250.

Morris, L. and Cilliers, T. 2005. Viral structure, replication, tropism, pathogenesis and natural history, in HIV/AIDS in South Africa. Ed. by Karim, S.S.A. and Karim, Q.A., Cambridge: Cambridge University Press, pp. 79 – 88.

Moye, J., Rich, K.C., Kalish, L.A., Sheon, A.R., Diaz, C., Cooper, E.R., Pitt, J. and Handelsman, E., for the Women and Infants Transmission Study Group. 1996. Natural history of somatic growth in infants born to women infected by human immunodeficiency virus. Journal of Pediatrics, 128(1):58 – 70.

Nielsen, K. 1999. Pediatric HIV Infection, Medical Education Collaborative.Medscape Inc. Available at www.medscape.com/viewprogram/671_pnt. [Accessed April 12, 2006].

O'Brien, W.A., Hartigan, P.M., Martin, D., Esinhart, J., Hill, A., Benoit, S., Rubin, M., Simberkoff, M.S. and Hamilton, J.D. 1996. Changes in plasma HIV-RNA and CD4 lymphocyte counts and the risk of progression to AIDS. Veteran Affairs Study Cooperative Group on AIDS. New England Journal of Medicine, 334: 426 – 431.

Oleske, J.M., Rothpletz-Puglia, P.M. and Winter, H. 1996. Historical perspectives on the evolution in understanding the importance of nutritional care in pediatric HIV infection. Journal of Nutrition, 126: 2616S – 2619S.

Omene, JA., Easington, C.R., Glew, R.H., Prosper, M. and Ledlie, S. 1996. Serum beta-carotene deficiency in HIV-infected children. Journal of the National Medical Association, 88: 789.

Pacht, E.R., Diaz, P., Clanton, T., Hart, J. and Gadek, J.E. 1997. Serum vitamin E decreases in sero-positive subjects over time. The Journal of Laboratory and Clinical Medicine, 130: 293.

Periquet, B.A., Jammes, N.M., Lambert, W.E., Tricoire, J., Moussa, M.M., Garcia, J., Ghisolfi, J. and Thouvenot, J.P. 1995. Micronutrient levels in HIV-1-infected children. Acquired Immune Deficiency Syndromes, 9(8): 887 – 893.

Piwoz, E.G. and Preble, E.A. 2000. HIV/AIDS and nutrition: A review of the literature and recommendations for nutrition care and support in Sub-Saharan Africa, Washington DC: SARA Project Bureau for Africa, Office for Sustainable Development, US Agency for International Development.

Pollack, H., Glasberg, H., Lee, H., Nirenberg, A., David, R., Krasinski, K., Borkowsky, W. and Oberfield, S. 1997. Impaired early growth of infants perinatally infected with human immunodeficiency virus: correlation with viral load. Journal of Pediatrics, 130: 915 – 922.

Powell, J., Yoshida, S.H., Van de Water, J. and Gershwin, M.E. 2001. The molecular and immunologic evaluation of nutritionally at-risk hosts, in Nutrition, Immunity, and Infection in Infants and Children. Ed. by Suskind, R.M. and Tontisirin, K., Nestle Nutrition Workshop Series, Pediatric Program, Vol.45, Nestec Ltd., Philadelphia: Vevy/Lippincott Williams and Wilkens.

Rabie, H., Marais, B.J. and Cotton, M.F. 2006. Preventing and diagnosing HIV infection in infants and children. Professional Nursing Today, 10: 33 – 36.

Ramakrishnan, U., Webb, A.L. and Ologoudou, K. 2004. Infection, immunity and vitamins, in Handbook of Nutrition and Immunity. Ed. by Gershwin, M.E., Nestel, P. and Keen, C.L., New Jersey: Humana Press, pp. 93 – 116.

Read, J.S., Bethel, J., Harris, D.R., Meyer, W.A., Korelitz, J., Mofenson, L.M., Moye, J., Pawwa, S., Rich, K. and Nugent, R.P. 1999. Serum vitamin A concentrations in a North American cohort of human immunodeficiency virus type1-infected children. Pediatric Infectious Disease Journal, 18: 134 – 142.

Reich, E.N. and Church, J.A. 1994. Oral zinc supplementation in the treatment of HIV-infection in children. Pediatric AIDS & HIV Infection, 5: 357 – 360.

Rodriguez, J.F., Cordero, J., Chantry, C., Gonzalez, S., Rivera, C., Febo, I. Colon, A. and Diaz, C. 1998. Plasma glutathione concentrations in children infected with human immunodeficiency virus. Pediatric Infectious Disease Journal, 17(3):236-241.

Rollins, N.C., Filteau, S.M., Elson, I. and Tomkins, A.M. 2000. Vitamin A supplementation of South African children with severe diarrhoea: optimum timing for improving biochemical and clinical recovery and subsequent vitamin A status. Pediatric Infectious Disease Journal, 19: 284 – 289.

Rothpletz-Puglia, P., Oleske, J., Boland, M., Banyai, J., D'Orlando, D. and Dieudonne, A. 1998. Prelimenary results of a retrospective study of nutrition intervention strategies for HIV-infected infants and children. International Conference AIDS, 1998; 12: 843 (abstract no. 42344), Available from http:/gateway.nlm.hih.gov/MeetingAbstracts/102231142.html. [Accessed September 21, 2006].

Rule, S.A.J., Hooker, M., Costello, C., Luck, W. and Hoffbrand, A.V. 2002. Serum vitamin B12 and transcobalamin levels in early HIV disease. American Journal of Hematology, 47: 167 – 171.

Salomon, J., De Truchis, P. and Melchior, J.C. 2002. Nutrition and HIV Infection. British Journal of Nutrition, 87: S111 – S119.

Semba, R.D., Ndugwa, C., Perry, R.T., Clark, T.D., Jackson, J.B., Melikian, G., Tielsch, J. and Mmiro, F. 2005. Effect of periodic vitamin A supplementation on mortality and morbidity of human immunodeficiency virus-infected children in Uganda: A controlled clinical trial. Nutrition, 21(1): 25 – 31.

Semba, R.D., Caiaffa, W.T., Graham, N.M.H., Cohn, S. and Vlahov, D. 1995. Vitamin A deficiency and wasting as predictors of mortality in human immunodeficiency virus-infected drug users. The Journal of Infectious Diseases, 171: 1196 – 1202.

Semba, R.D., Miotti, P.G., Chiphangwi, J.D. Liomba, G., Yang, L.P., Saah, A.J., Dallabetta, G.A. and Hoover, D.R. 1995. Infant mortality and maternal vitamin A deficiency during human immunodeficiency virus infection. Clinical Infectious diseases, 21: 966 – 972.

Semba, R.D. 2002. Vitamin A, carotenoids, and HIV infection, in Micronutrients in HIV Infection. Ed. by Friis, H., London: CRC Press, pp. 71 – 95.

Semba, R.D. and Tang, A.M. 1999. Micronutrients and the pathogenesis of HIV infection. British Journal of Nutrition, 81: 181 – 189.

Semba, R.D. 1997. Vitamin A and human immunodeficiency virus infection. Proceedings of the Nutrition Society, 56: 459 – 469.

Semba, R.D., Caiaffa, W.T., Graham, N.M.H., Cohn, S. and Valadof, D. 1994. Vitamin A deficiency and wasting as predictors of mortality in human immunodeficiency virus-infected injection users. The Journal of Infectious Diseases, 171: 1196 – 1202.

Shearer, W.T., Quinn, T.C., LaRussa, P., Lew, J.F., Mofenson, L., Almy, A., Rich, K., Handelsman, E., Diaz, C., Pagano, M., Smeriglio, V. and Kalish, L.A. 1997. Viral load and disease progression in infants infected with human immunodeficiency virus type 1. The New England Journal of Medicine, 336(19): 1337 – 1342.

Shearer, W.T., Easly, K.A., Goldfarb, J., Jenson, H.B., Rosenblatt, H.M., Kovacs, A. and Mcintosh, K. 2000. Evaluation of immune survival factors in pediatric HIV-1 infection. Annals of the New York Academy of Sciences, 918: 298 – 312.

Shisana, O. and Simbayi, L. 2002. Nelson Mandela/HSRC Study of HIV/AIDS - South African national HIV prevalence, behavioural risks and mass media household survey 2002. Cape Town: Human Sciences Research Council; 2002. Available from http://www.hsrcpress.ac.za/freedownload.asp?id=2009. [Accessed October 22, 2006].

Siberry, G.K., Ruff, A.J. and Black, R. 2002. Zinc and human immunodeficiency virus infection. Nutrition Research, 22: 527 – 538.

Simon, V., Ho, D.D. and Karim, Q.A., 2006. HIV/AIDS epidemiology, pathogenesis, prevention and treatment. The Lancet, 368: 489 – 504.

Sorensen, R.U., Butler, B. and Leiva, L.E. 2001. A review of the immune system: Methodology for evaluating the impact of nutrition on its function, in Nutrition, Immunity, and Infection in Infants and Children. Ed. by Suskind, R.M. and Tontisirin, K., Nestle Nutrition Workshop Series, Pediatric Program, Vol.45, Nestec Ltd., Philadelphia: Vevy/Lippincott Williams and Wilkens.

Steenkamp, L. and Visser. 2002. Can nutrition support prevent HIV disease progression and delay AIDS related mortality? The Specialist Forum, 2(5): 44 – 54.

Steenkamp, L. and Dannhauser, A. 2001. Nutritional management of the HIV/AIDS patient – A practical approach. The Southern African Journal of HIV Medicine, December issue: 31 – 36.

Steenkamp, L., von der Marwitz, J. and Giovanelli, C. 2004. Nutritional status of HIV+ pre-school children in South Africa – Summary of unpublished research. Field Exchange, Emergency Nutrition Network, Issue 22. Available from: http://www.enonline.net/fex/22/fex22.pdf [Accessed 2006 Oct 12].

Taha, T.E., Kumwenda, N.I., Broadhead, R.L., Hoover, D.R., Graham, S.M., Van der Hoven, L., Markakis, D., Liomba, G.N., Chiphangwi, J.D. and Miotti, P.G. 1999. Mortality after the first year of life among human immunodeficiency virus type 1-infected and uninfected children. The Pediatrics Infectious Disease Journal, 18: 689 – 694.

Tamura, J., Kubota, K., Murakami, H., Sawamura, M., Matsushima, T., Tamura, T., Saitoh, T., Kurabayshi, H. and Naruse, T. 1999. Immunomodulation by vitamin B12: Augmentation of $CD8^+$ T-lymphocytes and natural killer (NK) cell activity in vitamin B12-

deficient patients by methyl-B12 treatment. Clinics of Experimental Immunology, 116: 28.

Tanchou, V. Decimo, D., Pechoux, C., Lener, D., Rogemond, V., Berthoux, L., Ottman, M. and Darlix, J. 1998. Role of N-terminal zinc finger of human immunodeficiency virus Type 1 nucleocapsid protein in virus structure and replication. Journal of Virology, 72(5): 4442 – 4447.

Tang, A.M. and Smit, E. 2002. Vitamins C and E, and HIV infection, in Micronutrients in HIV Infection. Ed. by Friis, H., London: CRC Press, pp. 111 – 133.

Tang, A.M., Graham, N.M.H., Chandra, R.K. and Saah, A.J. 1997. Relation between serum vitamin B6, B12 and folate levels and human immunodeficiency virus type 1 (HIV-1) disease progression. Journal of Nutrition, 127: 345 – 351.

Tang, A.M., Lanzillotti, J., Hendricks, K., Gerrior, J., Ghosh, M., Woods, M. and Wanke, C. 2005. Micronutrients: current issues for HIV care providers. Acquired Immune Deficiency Syndromes, 19:847-861.

Tang, A.M., Graham, N.M. and Saah, A.J. 1996. Effects of micronutrient intake on survival in human immunodeficiency virus type 1 infection. American Journal of Epidemiology, 143(12): 1244 – 1256.

Tang, A.M., Graham, N.M.H., Semba, R.D. and Saah, A.J. 1997. Association between serum vitamin A and E levels and HIV-1 disease progression. Acquired Immune Deficiency Syndromes, 11: 613 – 620.

Thorne-Lyman, A., Jackson, R. and Erdelmann, F. 2004. Exploring opportunities for food and nutritional support of HIV/AIDS affected individuals and households in emergency settings. Abstract, eJournal of the International AIDS Society. Available from www.ias.sa/ejias/show.asp?abstract_id=2169195 [Accessed September 9 2004].

Tomkins, A.M. 2005. Evidence-based nutrition interventions for the control of HIV/AIDS, South African Journal of Clinical Nutrititon, 18(2): 187 – 191.

Tomkins, A. 1981. Nutritional status and severity of diarrhoeal disease among preschool children in rural Nigeria. The Lancet, I: 860 – 862.

UNAIDS/WHO. 2002. Improving estimates and projections of HIV/AIDS, Report of a meeting of the UNAIDS/WHO Reference group for "Estimates, Modelling and Projections" held at San Lorenzo de El Escorial, December 4 – 6th 2002.

United Nations System: Standing Committee on Nutrition. 2004. 5th Report on the World Nutrition Situation: Nutrition for improved development outcomes, March 2004.

Villamor, E., Misegades, L., Fataki, M.R., Mbise, R.L. and Fawzi, W.W. 2005. Child mortality in relation to HIV infection, nutritional status, and socio-economic background. International Journal of Epidemiology, 34(1): 61 – 68.

Visser, M. 2005. Nutritional prophylaxis, in HIV/AIDS in South Africa. Ed. By Karim, S.S.A. and Karim, Q.A., Cambridge: Cambridge University Press, pp. 463 - 476.

Visser, M.E., Maartens, G., Kossew, G. and Hussey, G.D. 2003. Plasma vitamin A and Zinc levels in HIV-infected adults in Cape Town, South Africa. British Journal of Nutrition, 89: 475 – 482.

WHO, 2006. Antiretroviral therapy of HIV infection in infants and children in resource-limited settings: towards universal access – Recommendations for a public health approach, World Health Organisation, Geneva, Switzerland.

WHO, 2003. Nutrient requirements for people living with HIV/AIDS. Report of a technical consultation. World Health Organisation, Geneva, 13 – 15 May 2003, Geneva, Switzerland.

Wood, R. 1999. Following the HIV-1 trail – from source to South Africa. South African Medical Journal, 89(11): 1146.

Woollard, I., Unpublished report on poverty in South Africa, 2003.

CHAPTER 3

METHODOLOGY

3. 1 Introduction

In this chapter a description of the operational definitions, population and sampling, choice and standardisation of methods, measuring techniques, apparatus, validity and reliability, and study procedures will be discussed. Methods for statistical analysis as well as a motivation for the development of the experimental product are also included.

This study formed part of a larger study in which four researchers participated. This researcher was responsible for development and coordination of the “larger-study” design. The role of this researcher in this study was summarised in Chapter 1.4.

3.2 Study design

This study was conducted in the form of a double-blinded, clinical, controlled, randomised prospective trial. Figure 3.1 shows the framework for the different variables used to determine growth, immune, antioxidant and health status in HIV-infected children.

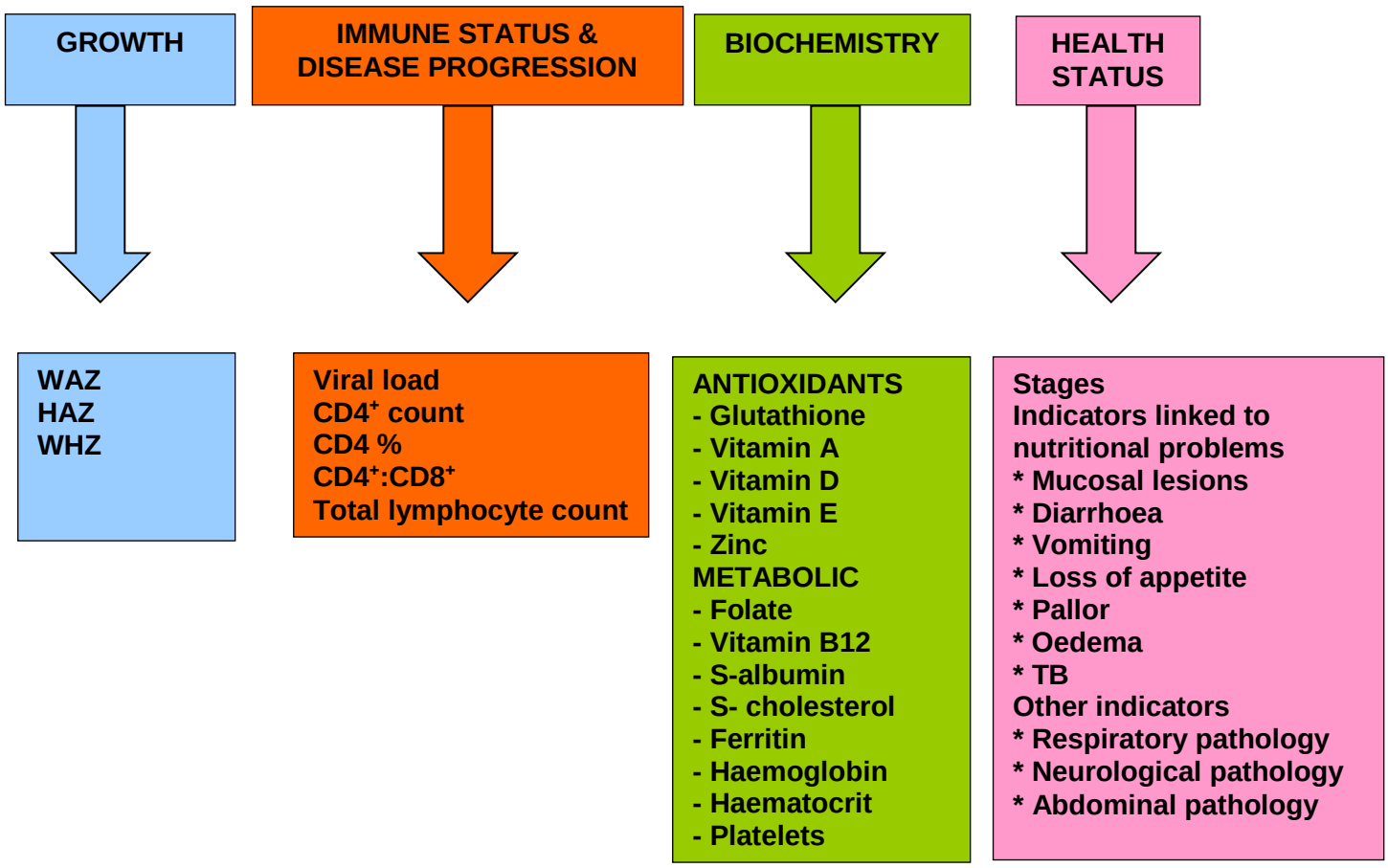

Figure 3.1: Framework to determine growth, immune status, biochemistry and health status in HIV-infected children in care centres

3.3 Study population

The study was conducted in six care centres in the Mangaung area of Bloemfontein. As a result of the ever increasing number of AIDS orphans, who may or may not be infected with the virus themselves, care centres linked to the government or non-governmental organisations are faced with the challenge of caring for these children. No specific recommendations or guidelines regarding nutrition support for this particular group are available.

Thirty seven antiretroviral naïve HIV-infected children, as determined by ELISA, older than 18 months, cared for at Lebone House and HIV/AIDS centres managed by Sunflower House in Mangaung, were included in the study (Table 3.1). The viral load done at baseline served as confirmation of the diagnosis.

Table 3.1: Summary of screening process for HIV in children in care centres in Mangaung

Name of Centre	Children tested (ELISA)	Children HIV-infected
Lebone House	70	20
Sunflower Day Care Centres:4 (all financed and supervised by Sunflower House)	70	12
Sunflower House	15	5
Total	155	37

Other benefits from selecting children from this institutionalised population included:

- Elimination of food insecurity issues that could impact on the intervention (any hungry child may benefit from supplementation and at these centres all the children received regular meals);
- Restriction of drop-outs (children had a smaller chance of not returning for follow up visits as all were registered to attend these centres);
- Regular health screenings and prevention of misuse of supplements by other family members (children received the supplemental meal instead of breakfast – 4 days per week – under supervision; intake was monitored);
- Specific recommendations regarding nutrient intake of HIV-infected children in care centres that can be provided to the authorities.

After collection of the baseline data, the sample was stratified according to nutritional status, $CD4^+$ count and/or CD4 percentage, presence of TB, the place of residence and whether they were permanent residents or only attending the day care centre. The children were then randomly placed in either the Experimental (E) group or the Placebo (P) group, by the Department of Biostatistics, Faculty of Health Sciences, University of the Free State. The researchers were blind to the randomisation process.

Both groups were supplemented with 150g (dry weight) of the experimental product (enzyme-modified enriched supplement) or placebo product (instant enriched mealiemeal) on a daily basis for four days per week. Both products were identical in macro and micronutrient content except for the added enzymes, ascorbic acid and organic sulphur in the experimental product. Staff at the centres were trained by a research assistant (Researcher 3), with the aid of a training manual (Appendix D). Monitoring was done in order to ensure that the applicable children receive the correct supplements and quantities (3.9 Study procedures).

3.3.1 Size

Initially, the researcher planned to include 70 HIV-infected children in the study. The care centres earmarked for this study, all indicated that they are focusing on care and support of HIV-infected children. However, from a total number of 70 children affected by HIV/AIDS in Lebone House and a total of 85 children in the five centres linked to Sunflower House (see Table 3.1), only 37 children had an initial positive ELISA, and were thus included in the study. It is therefore evident that the majority of children receiving care and support from these institutions are affected by HIV/AIDS and not infected as assumed before the start of the project.

3.3.2 Inclusion criteria

The following children were included in the study:

- HIV-infected children in centres as described in Table 3.1;
- Children older than the age of 18 months with a positive ELISA or known to be HIV-infected if younger than 18 months.

3.3.3 Exclusion criteria

The following children were excluded from the study:

- HIV negative children
- Children not up to date with the immunisation programme
- Children with known allergies for soy
- Children with foetal alcohol syndrome or other chronic illness
- Children referred for ARV treatment
- Children without consent from the legal guardian (see consent form Appendix B)

Children who refused to eat the supplements as well as drop-outs were recorded and documented. The 37 HIV-infected children included in the study were randomised; 19 children were included in the E group and 18 in the P group. Only 29 children completed the intervention. Eight children dropped out (2 deceased; 1 hospitalised; 1 refused the supplement; 2 presented with chronic diarrhoea; 1 was adopted; 1 only attended day care intermittently).

3.4 Ethical aspects

The study was approved by the Ethics Committee of the Faculty of Health Sciences, University of the Free State (ETOVS 190/00). All forms used for data capturing only referred to record numbers with no reference to personal information of the participants. For illiterate guardians, the information was explained in their home language and the form was signed by two witnesses. All mothers or legal guardians in the case of the orphans, gave written consent for the initial HIV testing and received counselling before and after the test. All mothers or guardians of HIV-infected children, who agreed that their children could participate in the intervention, signed consent forms (Appendix B).

Both groups received the same amount of macronutrients as well as micronutrients to ensure that no individual patient taking part in the study was affected in a negative manner. The experimental and placebo products were of high quality and the micronutrient levels in both products exceeded the levels available in products on the existing Free State protocol, as well as the micronutrient levels in the multivitamin syrup available for distribution. It was decided that if the outcome of the study indicated that children in both groups experienced significant improvements in growth, immune, micronutrient and health status, the supplements would be provided to them free of charge until alternative arrangements could be made.

3.5 Piloting

Before the start of the main study, a pilot study was done on 17 children at Oreratile Adra Care Centre for a period of four days. The latter is a centre caring for children affected or infected with HIV/AIDS in Mangaung, but could not be considered for the

study as another nutrition intervention project was due to start within a three month period after the pilot study. The aim of the pilot study was to test the acceptability of the two supplements, the ease with which the supplements were mixed, the ease with which the questionnaires were handled and understood and the testing of techniques for accumulation of anthropometrical data, after which final changes to questionnaires were done and training manuals compiled. Children were given 150 g (dry weight) or 450 g (reconstituted) of the experimental product for two days and then crossed over to the placebo product for two days. Leftovers were weighed with an electronic scale. Results indicated that children consumed a mean portion of 376 g of the placebo product and 415 g of the experimental product. None of the children in the pilot study refused either of the supplements, indicating that both supplements were acceptable.

3.6 Variables

Variables defined to meet the aim and objectives of this study, included growth, immune status, disease progression, biochemical and health status variables as described in 3.2 (Figure 3.1).

3.6.1 Growth

For the purpose of this study growth refers to the change between the baseline and end values of weight (kg) and height (cm). These were compared with the norms for physical growth of the National Centre for Health Statistics and CDC data. Z-scores which reflect the standard deviations from the median value of the reference data were calculated to determine:

- Baseline growth as reflected by WAZ, HAZ and WHZ.
- Growth as indicated by differences in WAZ, HAZ and WHZ between baseline and end of study;
- Weight growth velocity (g/kg/day gained).

The most commonly used cut-off with Z-scores is -2 standard deviations (SD) for moderate depletion and -3 SD for severe depletion. Children with Z-scores below -2 for WAZ, HAZ and WHZ were considered moderately underweight, stunted and wasted, while those with a Z-score below -3 were considered severely malnourished (Torun, 2006, p.890; Cogill, 2003). For stratification purposes children with a WAZ below -2 SD were classified in a separate group from those with a WAZ equal or above -2 SD, before they were randomised.

3.6.2 Immune status

Immune status for the purpose of this study, refers to the $CD4^+$ cell count, CD4 percentage and CD4:CD8 (Martin, 2000).

Immune status changes were defined and classified according to changes in the:

- number (cells/ mm^3) of $CD4^+$ cells;
- percentage of $CD4^+$ cells (Table 2.1);
- CD4:CD8; and
- total lymphocyte count.

3.6.3 Disease progression

Disease progression, although linked to the immune status, for the purpose of this study, refers to viral load (Martin, 2000). Viral load was indicated by the number of HIV-1 RNA copies/ml. Changes in disease progression were indicated by changes in the viral load.

3.6.4 Biochemical status

Biochemical status for the purpose of this study, refers to certain micronutrients, biochemical and haematological values. All the assays were performed by Voigt & Partners, Pathcare, Bloemfontein and normal reference ranges were thus obtained from that particular laboratory. Haematology cut-off values were adapted by the laboratory from Nathan and Oski's Haematology of Infancy and Childhood (Nathan et al., 2003). The normal reference ranges for the biochemical and haematological indicators can be classified as follows:

3.6.4.1 Antioxidants

- Glutathione: 24 – 37 mg/dL
- Vitamin A: 20 – 43 µg/dL
- Vitamin D: 20 – 60 ng/ml
- Vitamin E: > 6 mg/L
- Zinc: 12 – 17 µg/L

3.6.4.2 Metabolic indicators

- Serum albumin: 37 – 52 g/L
- Serum cholesterol: 3 – 5 mmol/L
- Serum ferritin: 18 – 67 ng/mL
- Haemoglobin deficient: less than 11 g/dl (children below 5 years)
- Haemoglobin deficient: less than 11.5 g/dl (children between 5 and 11 years)
- Haematocrit: 0.35 – 0.45 l/l
- Platelets: 140 – 420 x 10^9/L
- WBC: 4.5 – 13.5 x 10^9/L
- Folate: > 12.19 nmol/L
- Vitamin B12: 156 – 672 pmol/L

3.6.5 Health status

Health status refers to the clinical stage, presence of certain symptoms and other indicators and were categorised as follows:

- Clinical stages according to the WHO classification (WHO, 2006)
- The presence of symptoms linked to nutritional problems
    - Mucosal lesions
    - Diarrhoea
    - Vomiting
    - Loss of appetite
    - Pallor

- Oedema
- TB

- Other indicators
  - Respiratory pathology
  - Neurological pathology
  - Abdominal pathology

3.7 Measuring instruments and techniques

All instruments, techniques and procedures used in this study were standardised prior to implementation. Methods were also standardised to ensure validity and reliability.

3.7.1 Anthropometry

Weight and height measurements were obtained by a trained fieldworker (Researcher 2) using standardised techniques. The data collection process was blind as the fieldworker was unaware of the supplementation (E or P) group the children belonged to. A SECA electronic scale (model 708) was placed on a flat, hard surface and a trained dietitian/fieldworker determined the weight of children to the nearest 0.1 kg. Children were measured without shoes in only light clothing and had to stand independently in the middle of the platform with the body weight evenly distributed on both feet. The height of children older than 24 months, was obtained by means of a stadiometer. The feet and legs were correctly positioned (placing the child's feet flat and together in the centre of and against the back and base of the measuring frame, straightening the child's ankles and pushing the knees against the frame); with the child's shoulders level, hands at the

child's side and the line of sight level with the ground. The headpiece was then lowered through the hair and the measurement obtained to the nearest 0.1 centimeter. The recumbent length of children younger than 24 months was obtained by standardised methods. The accuracy and repeatability was ensured by taking and writing down three measurements of which the middle value was translated onto a spreadsheet. Reference standards by the US National Centre for Health Statistics (NCHS), were subsequently used to determine Z-scores for weight-for-age, height-for-age and weight-for-height (Cogill, 2003; Lee & Nieman, 2003).

3.7.2 Haemotologic and biochemical tests

Fasting blood samples of the children were collected by a medical doctor from the antecubital vein or failing that, the femoral vein. The area was disinfected with alcohol swabs before collection of the samples. Three 5 ml EDTA samples (Vacutainer) were taken, of which two had been wrapped in foil, to protect it from light, and stored on ice immediately after the collection process. A sample for one 10 ml clotting tube was collected as well. The lymph subset and viral load were measured according to standard techniques (Table 3.2).

3.7.2.1 Immune status/disease progression

The viral load indicates the amount of actual HIV in the body and is expressed as the number of HIV-1 RNA copies per milliliter. Viral load was determined at baseline and at the end of the intervention period. According to Martin (2002), changes in the viral load of more than 0.5 log can be viewed as significant, while change of 1 log or more can be interpreted as clinically significant.

The CD4 percentage and CD4:CD8 were determined by the reference laboratory using standardised methods. The $CD4^+$ count or percentage is used as indicator depending on the age of the child and it is done according to the 1994 Revised Pediatric HIV Classification System (CDC, 1994). End $CD4^+$ cell counts and percentages were compared to baseline values to determine changes over time.

Table 3.2: Description of measurements of immune parameters as supplied by Pathcare Pathologists

Parameter	Units	Method	Distributors	Catalogue no.
Viral load	HIV-RNA copies/ml	COBAS AMPLICOR Analyzer	Roche Diagnostic System Inc.	n/a
Lymph subset	Cells/mm^3	Flow cytometry	BD Bioscience	342447

3.7.2.2 Biochemical indicators

Methods for analysis of the antioxidants and other metabolic parameters are summarised in Tables 3.3 and 3.4 respectively. Results were translated on to a spreadsheet for statistical analysis.

Table 3.3: Summary of methods for analysis of anti-oxidants (as obtained from Pathcare)

Parameter	Unit	Method	Distributor	Catalogue no.
Glutathione	mg/dL	Spectrophotonome-trically	Calbiochem, United Kingdom	35412Y
Vitamin A	µg/dL	High Performance Liquid Chromatography	n/a	n/a
Vitamin D	ng/ml	Radioactive binding protein assessment	n/a	n/a
Vitamin E	mg/l	High Performance Liquid Chromatography	n/a	n/a
Zinc	µg/L	Hitachi Analyzer	Randox Laboratories Ltd., Crumlin, N. Ireland, UK	ZN 2341

Table 3.4: Summary of methods for analysis of metabolic parameters (as obtained from Pathcare)

Parameter	Unit	Method	Distributor	Catalogue no.
s-albumin	g/L	Bichromatic Digital Endpoint	Beckman	467858
s-cholesterol	mmol/L	Enzymatic colometric assay	Beckman	467825
s-ferritin	ng/ml	Chem-iluminessence	Bayer	110746
Haemoglobin	g/dL	SLS Photometric	Roche	904-1141-4
Haematocrit	l/l	Impedance Photometric	Roche	834-0011-6
Platelets	$X10^9$/l	Impedance Photometric	Roche	834-0011-6
s-folate	nmol/l	Chem-iluminessence	Bayer	118551
s-vitamin B12	pmol/l	Chem-iluminessence	Bayer	110748

Laboratory quality control was performed using standard calibration and quality control specimens as supplied by the manufacturers of the respective methods.

3.7.3 Clinical features

Pediatric morbidity was monitored by a trained medical doctor (Researcher 4). Illness specifically thought to be linked with increased metabolic needs, interference with intake and absorption problems was monitored once per month during a clinical examination by a trained medical doctor/fieldworker. All symptoms were recorded on a standardised questionnaire (Appendix C).

Using the WHO classification (WHO, 2006) as basis, the following indicators were monitored twice per month during a medical examination:

- Medication administered;
- Temperature, pulse rate and respiratory rate;
- Prevalence of jaundice, pale pallor, clubbing, cyanosis, lymphadenopathy, oedema, skin rash, mucosal lesions, ear infection, loss of appetite, weakness, fatigue;
- Respiratory problems (dyspnea, tachypnea, crepitations, wheeze, TB);
- Abdominal pathology (diarrhoea, splenomegaly, hepatomegaly, vomiting, oral thrush);
- Neurological pathology (myopathy, neck stiffness, peripheral neuropathy, encephalopathy).

The envisioned goal was initially to determine cognitive impairment (as assessed with tools developed by Occupational Therapy Department – Free State University). The age range of the children and specifically the high number of children above the age of six years, made such an evaluation impossible as the tool only applied to children below the age of 60 months.

3.8 Development of product

As summarised in Chapter 2, instant fortified soy/maize supplements have been in use for a number of years in most of the provinces as part of the health facility based programme. Some of the maize/soy blends have come under scrutiny due to low bioavailability of iron and zinc, due to high concentrations of phytic acid. Mineral bioavailability in cereal-based complementary foods, can, however, be improved by reducing the content of phytic acid, or by promoting the intake of enhancers, for example ascorbic acid (Gibson et al., 1998).

The presence of infection (eg. HIV/TB) may also cause loss of appetite, which can lead to a decrease in food and energy intake. The total solids content of a diet based on starch-containing foods may be limited by the resulting thickness or viscosity of the cooked product. The viscosity of such a mix can be reduced without sacrificing its energy density by adding an exogenous source of amylase to liquefy the gelatinised starch. Several studies (Den Besten et al., 1998; Gopaldas & Chinnama, 1992) proved that the addition of amylase to a starch based diet can significantly increase energy intake in children.

The researcher requested Diva Nutritional Products (Pty) Ltd. to adapt one of the enriched maize supplements by incorporating some of the findings as discussed (the addition of enzymes and additional ascorbic acid) into this alternative product – the experimental product.

3.8.1 Product verification

Diva Nutritional Products (Pty) Ltd. manufactured and packed the experimental product as well as the placebo, after which they were labeled with either a red or blue sticker. Until the collection of the end values, only the manufacturer knew which of the two products contained the enzymes. The products were provided to the institutions free of charge for use in the clinical trial. The vitamin/mineral mixtures for both the test product and the standard enriched mealiemeal were bought as a pre-mix from Roche Laboratories and added to the soy-maize blend at the factory. Two markers in both products were analysed by an independent laboratory (SABS analysis) to ensure that micronutrient values corresponded to the specification.

3.8.2 Nutrition analysis

The experimental product as well as the placebo contained approximately 13.5 percent of total energy from protein, 52 percent from carbohydrates and 34.5 from percent fat – thus reflecting a typical macronutrient distribution of the usual diet the children received in the care centres. The complete nutrition analysis is summarised in Table 3.5

Table 3.5: Nutritional analysis of experimental product and placebo (SABS analysis)

Nutritional content	***Amount per 100 g***	
	Experimental	***Placebo***
Protein (g)	13.9	13.9
Total Carbohydrate (g)	53.5	50.5
Fat (g)	15.7	15.7
Energy (kJ)	1727	1727
Isoflavones (mg)	94	94
Vitamin A (mcg RE)	600	600
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 B 12 (mcg)	2.5	2.5
Biotin (mcg)	50	50
Pantothenic acid (mg)	4	4
Vitamin K (mcg)	30	30
Sulphur (mg)	**34**	**0**
Calcium (mg)	825	825
Phosphorus (mg)	660	660
Potassium (mg)	550	550
Iron (mg)	10	10
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	**+**	**-**

3.9 Study procedures

An outline of the study procedures is described in Figure 3.2.

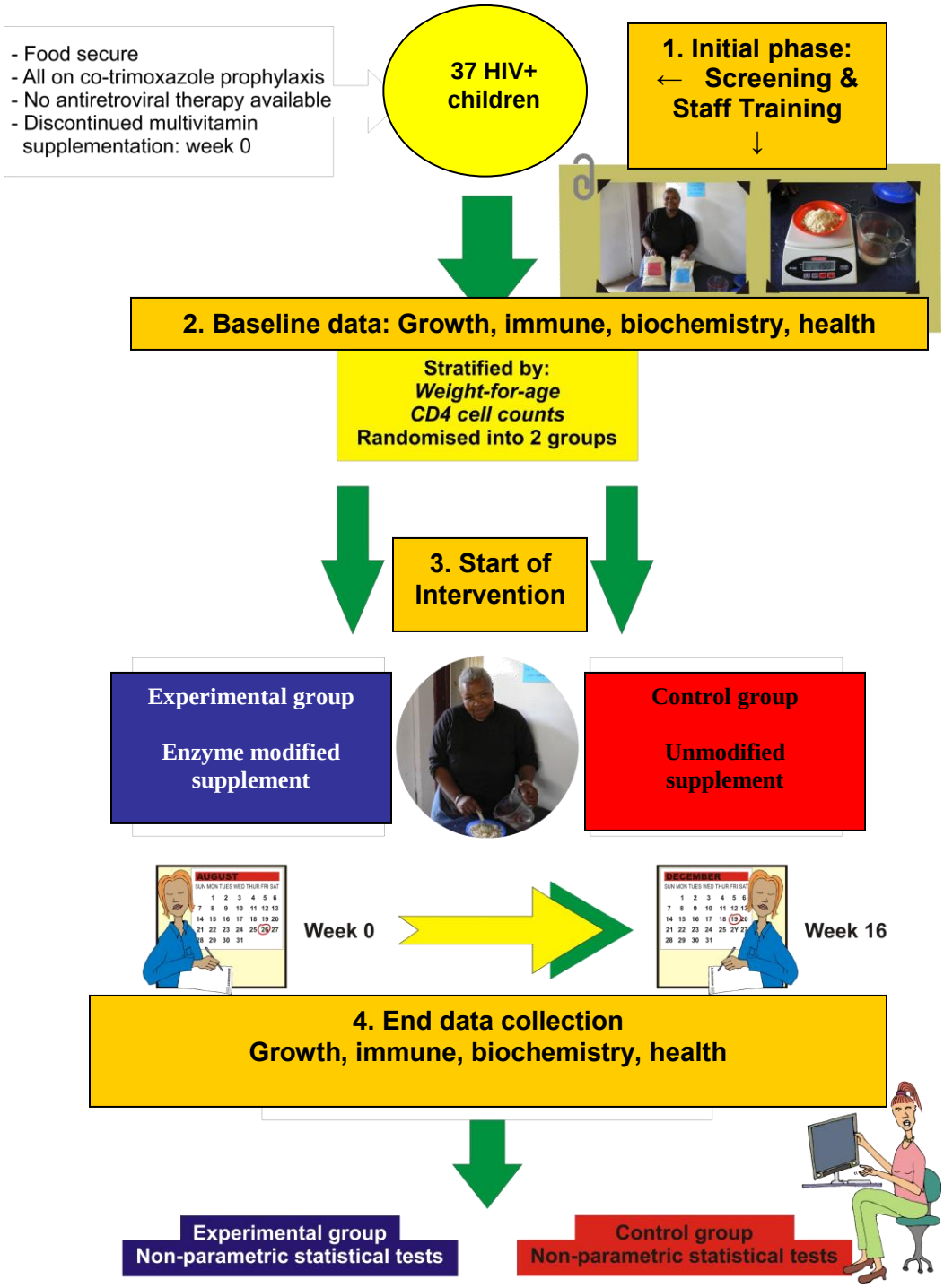

Figure 3.2 Illustration of study procedures and phases

The study was divided into four phases, i.e. the initial phase, the baseline data collection, start of the intervention with quality control and the end data collection.

3.9.1 Initial phase

The initial phase of the study can be summarised as follows (Figure 3.3)

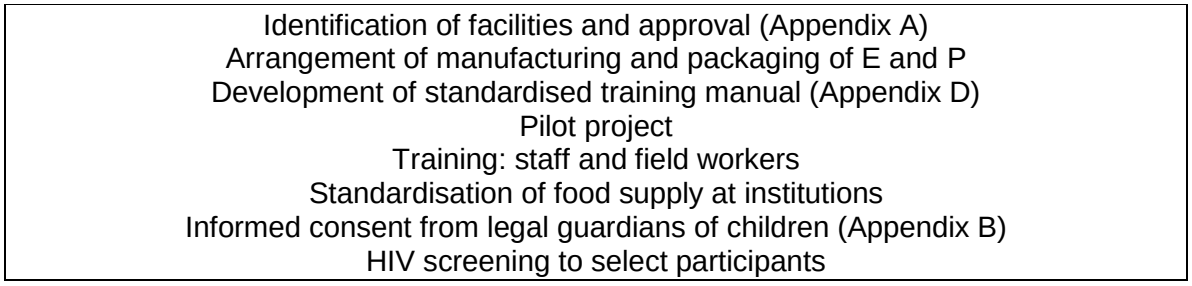

Figure 3.3: Summary of the initial phase of the study

3.9.2 Baseline data collection

At baseline, the trained researcher and fieldworkers (Researchers 2 and 4) collected the baseline data for the growth, immune, biochemical and health status. The children were then stratified and randomly divided into the Experimental and Placebo groups by a biostatistician from the Department of Biostatistics, Free State University.

3.9.3 Intervention, monitoring and quality control

The experimental or placebo product was provided to all HIV-infected children in the E and P groups respectively. Both E and P provide the same amount of energy, macronutrients, and micronutrients with the only modifications regarding the addition of enzymes, ascorbic acid, organic sulphur and NAC in the experimental product (Table 3.5).

During weekends no supplementation was provided as some of the children went home and researchers would have had little or no control over whether the children consumed the products or not.

Only the manufacturer of the supplements knew which of the two products contained the enzyme mixture. To ensure that children in the E and P groups received the correct supplements respectively, colour coding was used. The experimental and placebo products were packed in plastic bags with two different colours, mixed in two bowls of the same colours and dished up in cups or bowls of the same colours.

A fieldworker (Researcher 3) visited the two institutions on a daily basis to weigh the supplements (E and P) before and after consumption. This fieldworker (Researcher 3) was also responsible for the quality control of the study; to ensure that every child received and ate the correct type (experimental or placebo) and amount of supplement.

3.9.4 End data collection

After a period of 16 weeks of intervention, this researcher and the fieldworkers (Researchers 2, 3 and 4) collected the end data, which entailed precisely the same procedure as during the baseline data collection.

3.10 Statistical analysis

The statistical analysis was done by the Department of Biostatistics at the University of the Free State. Data was processed using SAS. Descriptive statistics including frequency distribution was used to summarise the categorical data. The distribution of

most of the numerical variables was skewed. It was therefore decided to use the percentiles to summarise numerical variables. Nonparametric statistical methods (Mann-Whitney and Kruskall-Wallis tests; Spearman correlation coefficients) were used to compare within variables between subgroups. Epi Info was used to calculate z-scores and percentiles of anthropometric data.

Growth, immune, biochemical and health parameters after 16 weeks into the study, were compared to baseline values in order to determine whether any significant changes took place, using 95 % confidence intervals.

3.11 Summary

The aim of this study was to determine the impact of an enzyme modified, enriched maize supplement on the growth, immune and health status of HIV-infected children in care centres in Mangaung. The determination of the baseline nutritional, immune, antioxidant and health status were sub-objectives of the study.

Nutritional indicators or growth was described by WAZ, HAZ and WHZ and changes during the intervention period. Immune status was determined by CD4 levels and viral load. Antioxidants included serum levels of vitamin A, vitamin D, vitamin E, zinc and glutathione. Health status was determined by clinical features (stages and symptoms) as well as certain biochemical indicators.

All measurements were done by trained fieldworkers using standardised methods to ensure reliability and validity of data. Reliability was improved by using trained fieldworkers and ensuring that the same fieldworker did repeated measurements at

baseline and at the end of the study. Validity was improved by using standardised techniques and controlling that the supplements were consumed during the intervention period. Problems encountered and steps taken to overcome these problems will be described in Chapter 8.

3.12 References

Centre for Disease Control and Prevention (CDC). 1994. 1994 Revised classification system for HIV infection in children less than 13 years of age. Official authorized agenda: Human immunodeficiency virus infection codes and official guidelines for coding and reporting ICD-9-CM, Morbidity and Mortality Weekly Report, 43(RR-12): 2-3.

Cogill B. 2003. Anthropometric Indicators Measurement Guide. Food and Nutrition Technical Assistance. 2003 edition, pp. 17 - 38. Available from: http:/www. Fantaproject.org/publications/anthropom.shtml, [Accessed 2004 August 15].

Den Besten, L., Glatthaar, I.I. and Ijsselmuiden, C.B. 1998. Adding α-amylase to weaning food to increase dietary intake in children. A randomised controlled trial. Journal of Tropical Paediatrics, 44: 4 – 6.

Gibson, R.S., Ferguson, E.L. and Lehrfeld, J. 1998. Complementary foods for infant feeding in developing countries. European Journal of Clinical Nutrition, 52: 764 – 770.

Gopaldas, T. and Chinnamma, J. 1992. Evaluation of a controlled 6-month feeding trial on intake by infants and toddlers fed a high energy-low bulk gruel versus a high energy-

high bulk gruel in addition to their habitual home diet. Journal of Tropical Paediatrics, 38: 278 – 283.

Lee, R.D. and Nieman, D.C. Nutritional Assessment, Third Edition, New York, McGraw Hill Companies, 2003.

Martin, D. 2002. Laboratory monitoring of HIV. Southern African Journal of HIV Medicine, July issue: 38 – 39.

Martin, D. 2000. Appropriate laboratory monitoring of HIV. South African Medical Journal, 90(1): 33 – 35.

Nathan, D., Orkin, S., Look, A.T. & Ginsburg, D. 2003. Nathan and Oski's Hematology of Infancy and Childhood. 6th ed., Philadelphia, WB Saunders.

Torûn, B. 2006. Protein-Energy Malnutrition, in Modern Nutrition in Health and Disease. Ed. by Shils, M.E., Shike, M., Ross, A.C., Caballero, B. and Cousins, R.J. 10th ed., Philadelphia, Lippincot Williams & Wilkins , pp. 880 – 908.

WHO, 2006. Antiretroviral therapy of HIV infection in infants and children in resource-limited settings: towards universal access – Recommendations for a public health approach, World Health Organization, Geneva, Switzerland.

CHAPTER 4

GROWTH, IMMUNE, MICRONUTRIENT AND HEALTH STATUS OF HIV-INFECTED CHILDREN IN CARE CENTRES IN MANGAUNG, SOUTH AFRICA

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Abstract

Aim: To assess the growth, immune, micronutrient and health status of antiretroviral naïve HIV-infected children in selected care centres in Mangaung, South Africa.

Method: A cross-sectional descriptive study was undertaken between September 2004 and March 2005 on antiretroviral naïve HIV-infected children in care centres in Mangaung.

Results: The study included 37 clinically stable and food secure HIV-infected children. Their median age was 5.35 years (ranging from 1.16 years to 10.17 years). Fifteen children (46%) were underweight, 30 children (77%) were stunted and one child was wasted. The median viral load (n=35) of the group was 117 000 copies/ml, the median $CD4^+$ cell count was 477 cells/mm^3 and the median CD4 percentage was 22.5%. Deficient serum levels relative to normal reference values were recorded for glutathione in 91%, albumin in 78%, vitamin A in 63%, vitamin D in 44%, zinc in 38% and vitamin E in 13%. Sixty percent of the children were anaemic and 30% were iron deficient. The most commonly occurring clinical features were lymphadenopathy in 84%, skin rashes in 51%, hepatomegaly in 32%, pallor in 41%, clubbing in 24%, ear infections in 24%, oedema in 22% and mucosal lesions in 16%. Only 8% presented with TB, while 19% had features of lower respiratory infection.

Conclusion: A high prevalence of chronic malnutrition and micronutrient deficiencies occurred among HIV-infected children residing in food secure care centres. The study highlights the importance of antiretroviral therapy and nutrition intervention in improving the management and prognosis of these children.

Introduction

It has been estimated that there are 5.5 million people living with HIV infection in South Africa, of whom 250 000 to 300 000 are children less than 15 years of age. The prevalence of HIV is highest among women of reproductive age i.e. 20 to 35 years, which places their offspring at particular risk of mother-to-child transmission, resulting in a public health concern.[1] In Africa, HIV-infection has reversed gains in child survival and lowered life expectancy.[2]

In HIV-infected children, acquired immunodeficiency syndrome (AIDS) is associated with an impaired nutritional status, growth failure and weight loss, as well as micronutrient deficiencies.[3] All these may further contribute to the already compromised immune system and lead to treatment failure.

According to data[4-6] on the nutritional status of HIV-infected children in South Africa, it has been estimated that approximately 25 to 30 % are underweight and 55 to 60 % are stunted. Hussey et al.[7] found in a group of 193 symptomatic HIV-infected children in Cape Town, that the weight-for-age of only 7.4 % was above the third percentile. However, in South African HIV-infected children, stunting is more prevalent in children above two years of age, compared to wasting. A high prevalence of multiple micronutrient deficiencies has also been found to affect more than 60 % of HIV-infected children.[4]

A number of non-governmental organisations in South Africa have been involved with the implementation of care centres for AIDS orphans, where these children have access to care and support, as well as regular meals – unlike many children staying in

the community. No information is, however, available regarding the nutritional and micronutrient status of these children.

The purpose of this study was to assess the growth, immune, micronutrient and health status of HIV-infected children in selected care centres in Mangaung, South Africa.

Methods

Study design and sampling

One hundred and fifty five food-secure HIV-affected children in care centres in Mangaung, South Africa, were screened to determine HIV status. Mangaung is in the central part of South Africa and most of the children are from an area within an urban township, which include formal and informal housing areas. Children known to be HIV-infected, as well as those with a positive result of ELISA (Enzyme-linked immunosorbent assay) for anti-HIV antibodies, were purposely sampled and recruited into the study between August 2004 and March 2005. A cross-sectional descriptive study was subsequently undertaken on 37 clinically stable, antiretroviral naïve HIV-infected children in these centres. Children with acute illness or episodes of hospitalisation in the preceding week were excluded from the study.

Growth

Growth was assessed by determining weight-for-age, height-for-age and weight-for-height z-scores for all the children and comparing it to the norms for physical growth of the National Centre for Health Statistics and CDC data. Anthropometric measurements were done by a trained dietitian using standardised techniques.[8] A SECA electronic scale (model 708) was used to determine the weight of the lightly

clothed children to the nearest 0.1 kg. A stadiometer was used to obtain the height of the children older than 24 months to the nearest 0.1 centimeter. In children younger than 24 months, the recumbent length was measured.

Immunological, metabolic and micronutrient status

*F*ollowing the nutritional and clinical assessment, fasting blood samples were collected by a trained medical doctor for the following investigations: $CD4^+$ cell count, viral load, full blood count, as well as serum levels of cholesterol, allbumin, glutathione, vitamin A, vitamin E, vitamin D, ferritin, zinc, folate and vitamin B12. The $CD4^+$ counts and percentages were used to classify children into immunological categories according to the Center for Disease Control (CDC) guidelines.[9]

The $CD4^+$ lymphocyte cell count was determined on EDTA-anti-coagulated whole blood samples using a flow cytometer. The plasma viral load (log_{10} HIV-1 RNA copies/ml) was quantified by means of a nucleic acid amplification test on a COBAS AMPLICOR (Roche Diagnostic System Inc.).

Plasma chemistry concentrations were quantified using serum prepared from whole blood clotted at room temperature. Serum albumin was measured by direct spectrophotometry, using a colorimetric assay. The serum total cholesterol was measured by means of an enzymatic colorimetric assay provided by Beckman. Vitamin A (retinol) and vitamin E were quantified by HPLC, and Vitamin D by radioactive binding protein assays. Ferritin, vitamin B12 (cobalamin) and folate were quantified by Chem – Iluminesence assays and zinc by flame atomic absorption spectrometry. Blood micronutrient concentrations of the population group were compared with local age-related normal values.

Health status

Health status was determined by means of a clinical examination undertaken by a trained medical doctor. The clinical examination included an assessment of the child's vital signs e.g. temperature, pulse rate, respiratory rate and an examination of all the systems: general, respiratory, cardiovascular, abdominal and neurological.

Ethical aspects

Written consent was obtained from the legal guardian of each child. The study was approved by the Ethics Committee of the Faculty of Health Sciences of the University of the Free State, South Africa.

Data entry and analysis

Frequencies and percentages were used to summarise the categorical data. The distribution of most numerical variables was skewed. It was therefore decided to use the percentiles to summarise numerical variables. Nonparametric statistical methods (Mann-Whitney and Kruskall-Wallis tests) were used to compare within variables between subgroups. All analyses were done using SAS. Epi Info was used to calculate z-scores and percentiles of anthropometric data. Correlations between age and viral load were determined with the Spearman rank correlation test, with a p-value of less than 0.05 considered to be significant.

Results

General and growth

The median age was 5.35 years (ranging from 1.16 years to 10.17 years), with 43 % (n=16) of the sample female and 57% (n=21) male. The median (quartiles) for the following indicators of growth were as follows: weight-for-age Z-score -1.97 (-2.48; -1.4), height-for-age Z-score -2.59 (-3.35; -2.03) and weight-for-height Z-score -0.66 (-0.85; 0.05). Fourteen children (37.8%) had an upper arm circumference below the fifth percentile, while only two (5.4%) had a triceps skinfold measurement below the fifth percentile. Stunting appeared to be a much bigger problem than underweight, with 76.7% (n=30) of the children with a height for age Z-score < -2, while 45.9% had a weight-for-age Z-score of < -2.

Immunological, metabolic and micronutrient status

The median (quartiles) viral load (n=35) was 117 000 copies/ml (14500; 305 000) and the median (quartiles) $CD4^+$ cell count (n=35) 477 cells/mm^3 (300; 791). The median (quartiles) $CD4^+$ percentage was 22.5% (14.2; 35.8). There was no significant correlation between the age of the children and the viral load in this study. A significant (p=0.04) but weak negative correlation (r=-0.35) was, however, demonstrated between the total number of T-lymphocytes and the age of the children. According to the CDC classification, 40% (14/35) of the group could be categorised in Category 1, with no evidence of immunosuppression. Within the group, 34.3% (12/35) were moderately immunosuppressed (Category 2) and a 25.7% (9/35) severely immunosuppressed (Category 3).

The percentages of children with abnormal values in terms of the metabolic indicators are summarised in Table 1. Median serum albumin levels were 32 g/l, which fell below the normal range of 37 to 52 g/l. Seventy eight percent of the group presented with low serum albumin levels.

Median serum ferritin and white blood cells were within the normal ranges for these variables, but median haemoglobin (10.8 g/dL) and haematocrit (0.33 l/l) concentrations were lower than the normal ranges. Of the total sample, 60% (n=21) were anaemic and 24% (n=11) were iron depleted. Of the anaemic group 38% (n=8) were iron depleted. Unfortunately red blood cell morphology was not available to determine iron deficiency anaemia.

The percentages of children with abnormal values in terms of micronutrients are summarised in Table 4.1. Laboratory error resulted in missing values for some of the variables. Of the children, low levels in relation to the normal reference range was found in 91.2% for glutathione, 62.5% for vitamin A, 43.8% for vitamin D, 38.2% for zinc and 12.5% for vitamin E.

TABLE 4.1: Percentage of HIV-infected children with micronutrient concentrations and metabolic parameters below or above the normal age-related range

Variable (n)	Normal range	Median concentration (quartiles)	Low values (frequency)		High values (frequency)	
			N	%	N	%
Zinc (34) µmol/l	12 – 17	12 (11; 14)	13	38.2	0	0
Glutathione (34) mg/dl	24 – 37	18 (15; 20)	31	91.2	0	0
Vitamin A (32) µg/dl	20 – 43	18.15 (15.3; 22.35)	20	62.5	0	0
Vitamin D (32) ng/ml	20 – 60	20 (16; 24.5)	14	43.8	0	0
Vitamin E (32) mg/dl	3 – 9	8.05 (6.7; 9.65)	4	12.5	0	0
Serum cholesterol (37) mmol/l	3 – 5	3 (2.5; 3.3)	18	48.7	0	0
Serum albumin (37) g/l	37 – 52	32 (28; 35)	29	78.4	0	0
Serum ferritin (37) ng/ml	12 – 55	24 (16; 40)	11	29.7	5	13.5
Vitamin B12 (37) pmol/ml	156 – 672	482.9 (424.7; 617.9)	0	0	7	18.9
Serum folate (37) nmol/ml	> 12.19	36.2 (28.4; 54.4)	0	0	0	0
Haemoglobin (37) g/dl	11.5 – 13.5	10.8 (9.4; 11.9)	21	60	0	0
Haematocrit (37) l/l	0.35 – 0.4	0.33 (0.3; 0.35)	22	66.7	0	0
WBC (35) x 10^9/l	5 – 15.5	7.54 (5.97; 10.87)	2	5.7	4	11.4
Platelets (35) x 10^9/l	140 – 420	316 (285; 443)	2	5.7	11	31.4

Health status

The most commonly occurring clinical features were lymphadenopathy (83.8%), skin rashes (51.4%) and pallor (40.5%) (Table 4.2). Signs of lower respiratory tract infection were present in seven (18.9%) of the children. Only three children (8.1%) presented with TB, with crepitations (43.2%) and hepatomegaly (32.4%) the most common respiratory and abdominal pathology present respectively. Only six children (16.2%) presented with diarrhoea, while splenomegaly, vomiting and oral thrush occurred in less than 10% of the sample. Myopathy and peripheral neuropathy were present in 37.8% and 35.1% respectively of the sample. No children presented with neck stiffness and encephalopathy.

According to the WHO Clinical Staging of HIV for Infants and Children with established HIV Infection, only four children could be classified as Clinical Stage 1, while seven fell into stage 2, seven into stage 3 and 19 into stage 4.

TABLE 4.2: Clinical features at baseline

Clinical sign	**N = 37**	**%**
Lymphadenopathy	31	83.8
Skin rash	19	51.4
Pallor	15	40.5
Hepatomegaly	12	32.4
Ear infection	9	24.3
Clubbing	9	24.3
Oedema	8	21.6
Signs of lower respiratory tract infection	7	18.9
Mucosal lesions	6	16.2
Diarrhoea	6	16.2
Splenomegaly	3	8.1
Oral thrush	2	5.4

Discussion

Pediatric HIV infection is associated with growth failure in most settings - even in children receiving antiretroviral drugs.[4,10] HIV-infected children in developing countries, and specifically South Africa,[4,6] experience more severe growth retardation than observed in HIV-infected children in developed countries.[11] A higher HIV-1 viral load has been associated with a greater risk of growth failure as well as poor linear growth in children.[12-14] Anthropometric results from this study seem to mirror these observations, except for the underweight and stunting being worse at 45.9% and 76.7% respectively. Several factors may contribute to growth failure in HIV-infected children. In more than one study,[13,15] growth failure has been linked to a decreased resting energy expenditure (REE) and total energy expenditure (TEE) – most likely as a result of deficient energy intake and a lower fat-free mass. In this study it was demonstrated that having access to regular, balanced meals in a care centre, clearly cannot undo the negative aspect of being an AIDS orphan without any immediate family. As HIV-infected infants with growth failure have as much as a fivefold increase in the risk of early death,[16] efforts to reverse growth failure in children attending care centres should receive priority, preferably by providing antiretroviral drugs and aggressive nutrition support to supplement the meals these children currently receive.

Apart from wasting, HIV infection also causes micronutrient deficiencies, which can further compromise the immune system, resulting in a poorer outcome.[17] Especially in Africa, deficiencies of a number of these micronutrients are more prevalent among children infected with HIV, than among HIV-negative children.[18] Results from this study have shown that micronutrient deficiencies occurred commonly in the majority of children, with the highest prevalence of deficiencies relating to glutathione, vitamin

A, zinc and vitamin D, which are all essential in maintaining the immune function. These results should, however, be treated with caution, as they are affected by the acute-phase response and thus are susceptible to changes in binding proteins.[19] Serum zinc and vitamin A levels normally decrease during acute phase response, while serum levels of ferritin increase. A small number (13.5%) of children had increased ferritin levels and as C-reactive protein levels were not available, serum levels of micronutrients in some children may not accurately reflect true micronutrient levels. This is supported by the fact the children with increased ferritin levels had significant decreases in both $CD4^+$ cell counts and serum zinc levels.

In terms of the clinical features of the children, a high prevalence of lymphadenopathy and skin rashes was observed, which is in keeping with other studies.[20] Respiratory pathology accounts for a significant proportion of illness in HIV-infected children. In this study the most common clinical sign of respiratory pathology was crepitations, while only three children (8.1%) had TB, which is much lower than figures reported in other studies.[21,22] In contrast to the hospitalised children in the study of van Gend et al.,[23] dyspnea and tachypnea were uncommon in the children included in this study. The most common clinical sign of abdominal pathology was hepatomegaly, with low percentages presenting with diarrhoea, vomiting and oral thrush, which could impact on food intake and absorption. Myopathy and peripheral neuropathy were present in approximately a third of the sample.

The limited sample size was one of the biggest limitations of the study. All care centres initially earmarked for the study indicated that their main focus was the care and support of HIV-infected children. However, of the more than 150 children in 6 centres, only 37 had a positive ELISA. It is therefore evident that the majority of the

children who are currently receiving care and support from institutions in this part of the country, are affected by HIV/AIDS and not infected as assumed before the commencement of the study.

The nutritional problems observed regarding underweight, stunting as well as the widespread micronutrient deficiencies seen in this group, emphasise the need to evaluate the impact of antiretroviral therapy on the growth of AIDS orphans as well as the impact of macro- and micronutrient supplementation as part of optimal management of children living with HIV/AIDS. This has long-term implications for these children with respect to their development, school achievement and economic productivity later.

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Disclosure

L Steenkamp is an independent Nutrition Consultant and has in the past provided consultancy services for Diva Nutritional Products.

References

1. Meyers T, Moultrie H, Sherman G, Cotton M, Eley B. Management of HIV-infected children. In: South African Health Review 2006, Durban, Health Systems Trust, 2006.
2. Cant AJ, Gibb DM, Davies EG, Cale C, Gennery AR. HIV Infection. In: McIntosh N, Helms P, Smyth R, editors. Forfar & Arneil's Textbook of Pediatrics. 6th ed. Spain: Churchill Livingstone, 2003; p. 1295 – 1309.

3. Miller TL, Evans SJ, Orav EJ, Morris V, McIntosh K, Winter HS. Growth and body composition in children infected with the human immunodeficiency virus. Am J Clin Nutr. 1993;57: 588 – 592.
4. Eley B, Sive A, Kossew G, Abelse L, Cooper M, Hussey G. Growth and micronutrient disturbances in stable, HIV-infected children in Cape Town. Ann Trop Paediatr. 2002;22: 19 – 23.
5. Eley B, Hussey G. Nutrition and human immunodeficiency virus infection in children. S Afr Med J. 1999; 89: 190.
6. Steenkamp L, von der Marwitz J, Giovanelli C. Nutritional status of HIV+ pre-school children in South Africa – Summary of unpublished research. Field Exchange, Emergency Nutrition Network. 2004. Issue 22. [Accessed 2006 Oct 12] Available from: http://www.enonline.net/fex/22/fex22.pdf
7. Hussey GD, Reijnhart RM, Sebens AM, Burgess J, Schaaf S, Potgieter, S. Survival of children in Cape Town known to be vertically infected with HIV-1. S Afr Med J. 1998; 88(5): 554 – 558.
8. Cogill B. Anthropometric Indicators Measurement Guide. Food and Nutrition Technical Assistance. 2003 edition. 2003 [Accessed 2004 August 15]. Available from: http:/www. Fantaproject.org/publications/anthropom.shtml
9. Centre for Disease Control and Prevention (CDC). 1994 Revised Classification System for HIV Infection in Children less than 13 years of age. Official Authorized Agenda: Human Immunodeficiency Virus Infection Codes and Official Guidelines for Coding and Reporting ICD-9-CM. Morbidity and Mortality Weekly Report. 1994; 43(RR-12): 2-3.
10. McKinney RE, Robertson WR and the Duke Pediatric AIDS Clinical Trials Unit. Effect of human immunodeficiency virus infection on the growth of young children. J Pediatr. 1993; 123: 579 – 582.

11. Carey VJ, Yong FH, Frenkel LM, McKinney RE Jr. Pediatric AIDS prognosis using somatic growth velocity. AIDS. 1998; 12: 1361 – 1369.
12. Chantry CJ, Byrd RS, Englund JA, Baker CJ, McKinney RE. Growth, survival and viral load in symptomatic childhood human immunodeficiency virus infection. Pediatr Infect Dis J. 2003; 22(12): 1033 – 1039.
13. Arpadi SM, Cuff PA, Kotler DP, Wang J, Bamji M, Lange M, Pierson RN, Matthews DE. Growth velocity, fat-free mass and energy intake are inversely related to viral load in HIV-infected children. J Nutr. 2000; 130: 2498 – 2502.
14. Pollack H, Glasberg H, Lee H, Nirenberg A, David R, Krasinski K, Borkowsky W, Oberfield S. Impaired early growth of infants perinatally infected with human immunodeficiency virus: correlation with viral load. J Paediatr. 1997; 130: 915 – 922.
15. Miller TL. Nutritional Aspects of HIV-infected children receiving highly active antiretroviral therapy. AIDS. 2003; 17 (suppl 1): S130 – S140.
16. Berhane R, Bagenda D, Marum L, Aceng E, Ndugwa C, Bosch RJ, Olness K. Growth failure as a prognostic indicator of mortality in pediatric HIV infection. Pediatrics. 1997; 100(1): E7.
17. Steenkamp L, Visser M. Can nutrition support prevent HIV disease progression and delay AIDS related mortality?. The Specialist Forum. 2002;2(50): 44 – 54.
18. Fawzi W, Mbise R, Herzmark E, Fataki M, Herrera M, Ndossi G, Spiegelman D. A randomised trial of vitamin A supplements in relation to mortality among HIV-infected and uninfected children in Tanzania. Pediatr Infect Dis J. 1999; 18(127):127 – 133.
19. Macallan DC. Nutrition and immune function in human immunodeficiency virus infection. Proc Nutr Soc. 1999(58):127 – 133.
20. Bagenda D, Nassali A, Kalyessubula I, Sherman B. Health, neurologic, and cognitive status of HIV-infected, long-surviving, and antiretroviral-naïve Ugandan children. Pediatrics. 2006; 117(3): 729 – 740.

21. Villamor E, Misegrades L Fataki MR. Child mortality in relation to HIV infection, nutritional status and socio-economic background. Int J Epidemiol. 2005;34(1): 61 – 68.
22. Madhivanan P, Mothi SN, Kumarasamy N, Yepthomi T, Venkatesan C, Lambert JS, Solomon, S. Clinical manifestations of HIV-infected children. Indian J Pediatr. 2003;70(8): 615 – 620.
23. Van Gend CL, Haadsma ML, Sauer PJJ, Schoeman CJ. Evaluation of the WHO clinical case definition for pediatric HIV infection in Bloemfontein, South Africa. J Trop Pediatr. 2003; 49(3): 143-147.

CHAPTER 5

CORRELATIONS BETWEEN GROWTH, IMMUNE, MICRONUTRIENT STATUS AND METABOLIC INDICATORS IN HIV-INFECTED CHILDREN IN CARE CENTRES IN MANGAUNG, SOUTH AFRICA

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Abstract

Correlations between growth, immune, micronutrient status and metabolic indicators were investigated in antiretroviral naïve, HIV-infected children in care centres. Thirty seven clinically stable, food secure HIV-infected children, with a median age of 5.35 years, were included in a cross-sectional descriptive study. The median viral load of the group was 117000 copies/ml, with the $CD4^+$ cell count and percentage, 477 cells/mm^3 and 22.5% respectively. The group had a median weight-for-age Z-score (WAZ) of -1.97, height-for-age Z-score (HAZ) of -2.6 and weight-for-height Z-score (WHZ) of -0.66. Significant but weak positive correlations were demonstrated between HAZ and the CD4%, serum cholesterol and -albumin. Apart from a significant but weak positive correlation between the WHZ and glutathione, no other correlations could be demonstrated between micronutrient levels and growth. Viral load was significantly but weakly to moderately negatively correlated with the CD4:CD8, CD4%, $CD4^+$ cell count, haemoglobin, serum albumin, -cholesterol, -vitamin A, -vitamin D and -zinc. Significantly weak to moderate positive correlations were reported between CD4 concentrations and serum albumin, -hemoglobin, -cholesterol, -vitamin A, -vitamin D, -zinc and -glutathione. The results indicate that high viral loads and decreased CD4 concentrations in food secure children in care centres are linked to decreased serum levels of micronutrients, albumin and haemoglobin. Micronutrient deficiencies did not correlate with any of the growth markers. An increased CD4% is, however, linked with a higher HAZ. The study highlights the need for antiretroviral therapy and nutrition supplementation to improve the immune and nutritional status of children in care centres.

Introduction

Growth failure and wasting are common findings in HIV-infected children and has been shown to contribute to increased viral loads (Pollack *et al*. 1997). Even HIV-infected children in developed countries show an inverse relationship between the level of viral replication and growth (Arpadi *et al*. 2000). According to Berhane *et al*. (1997), HIV-infected children with inadequate growth have as much as a fivefold increase in the risk of early death.

In developing countries, the situation surrounding the prevalence of growth failure is far worse compared to developed countries. It is believed to be mainly as a result from increased vulnerability of children due to the high number of orphans, food insecurity and poor availability of antiretroviral therapy. In South Africa, stunting has been shown to occur in 55 to 60% of HIV-infected children with the prevalence of wasting 25 to 30% (Eley *et al*., 2002a). Apart from growth failure, severe immuno suppression is also associated with anaemia in children (Eley *et al*., 2002b). In HIV-infected adults, disease progression and mortality have been associated with decreased serum albumin levels (Feldman *et al.*, 2000). This result could not be reproduced in HIV-infected children in developed countries; even those presenting with growth failure (Henderson *et al.* 1997). Clinical data on the correlations between viral load and growth indicators in developing countries are, however, lacking, making comparisons to data from developed countries difficult.

Micronutrients play an important role in maintenance of normal immune function. Micronutrient deficiencies are associated with both malnutrition and the presence of HIV-infection, in adults as well as children (Garg and Miller, 1999). Micronutrient

deficiencies seem to affect more than 60 percent of HIV-infected children in South Africa (Eley *et al*. 2002a). Although micronutrient deficiencies in HIV-infected adult populations have been widely investigated (Tang *et al*. 2005), only a small number of studies have been done on HIV-infected children (Irlam *et al.*, 2006; Tomkins, 2005).

The largest number of completed micronutrient studies, to date, in HIV-infected children have investigated vitamin A deficiency. Vitamin A deficiency can be described as a common occurrence in HIV-infected children (Garg & Miller, 1999), and has been associated with higher infant mortality and growth failure (Semba *et al*., 1997).

Other micronutrient investigations done amongst HIV-infected children in developed countries, reported reduced glutathione levels in a small group (n=20) of HIV-infected children, especially those with growth failure (Arpadi *et al*., 2000). Although zinc deficiency has been documented in HIV-infected adults, results with children have proven to be contradictory. Some studies have shown increased levels of zinc, while others reported severe deficiencies (Garg & Miller, 1999; Henderson *et al*., 1997). It is, however, important to remember that, like with vitamin A, serum levels of zinc may decrease as a result of the acute phase response and results should be treated with caution (Keen *et al.*, 2004). Significantly lower vitamin E levels were reported (Mastroiacovo *et al.*, 1996; Periquet *et al*., 1995) in small groups of HIV-infected children in comparison to HIV-negative controls. According to available literature, no trials have been reported on levels of vitamin D in HIV-infected children.

HIV/AIDS is having a devastating impact on the livelihoods and food security of many affected people in developing countries. Micronutrient deficiencies therefore seem to be more prevalent in disadvantaged communities where staple diets are inadequate in both macro- and micronutrients (Visser, 2005). Tomkins (2005) suggested a number of social development and nutrition intervention strategies to benefit vulnerable children in developing countries with malnutrition. In South Africa, non-governmental organisations are playing a leading role in supporting vulnerable HIV-infected and affected children by means of care centres. In these centres the children have access to regular, balanced meals and the primary health care system, ensuring compliance to vaccination schedules, vitamin A supplementation, regular deworming and co-trimoxizole prophylaxis for HIV-infected children. During this study, these children in the selected care centres, however, did not yet have access to antiretroviral drugs.

This study was used to investigate the possible correlation between growth, immune, micronutrient status and metabolic indicators in antiretroviral naïve, clinically stable HIV-infected children in care centers in Mangaung, South Africa.

Methods

Study design and sampling

One hundred and fifty five food-secure HIV-affected children in care centres in Mangaung, in the central part of South Africa, were screened to determine HIV status. A cross-sectional descriptive study was subsequently undertaken on 37 clinically stable, antiretroviral naïve, HIV-infected children in these centres. Children older than 18 months, with a positive HIV enzyme-linked immunosorbent assay (ELISA), or those younger than 18 months known to be HIV-infected, were

purposely sampled and recruited into the study that were performed between August 2004 and March 2005. Children with acute illness or episodes of hospitalisation in the preceding week were excluded from the study. All the children received co-trimoxazole prophylaxis.

Anthropometry

Anthropometric measurements were done by a trained dietitian using standardized techniques (Cogill, 2003). A SECA electronic scale (model 708) was used to determine the weight of the lightly clothed children to the nearest 0.1 kg. A stadiometer was used to obtain the height of the children older than 24 months to the nearest 0.1 centimeter. In children younger than 24 months, the recumbent length was measured. The weight and height were compared with the norms for physical growth using the National Center for Health Statistics (NCHS) standards.

Biochemistry

Fasting blood samples were collected by a trained medical doctor. Samples were taken from the antecubital vein after the puncture site was cleaned with alcohol. Two 5 ml EDTA samples (Vacutainer) were wrapped in foil to protect it from light and stored on ice immediately after collection. For the purpose of this study, immune status referred to the viral load (copies of HIV per ml of blood) and the CD4 concentrations ($CD4^+$ cell count per mm^3 and %). The plasma viral load (log_{10} HIV-1 RNA copies/ml) was quantified by means of a nucleic acid amplification test on a COBAS AMPLICOR (Roche Diagnostic System Inc.). The $CD4^+$ lymphocyte cell count was determined on EDTA-anti-coagulated whole blood samples using a flow cytometer (BD Biosciences FACSCalibur). The $CD4^+$ counts and percentages were

used to classify children into immunological categories according to the Centre for Disease Control (CDC) guidelines (CDC, 1994).

Plasma chemistry was quantified using serum prepared from whole blood, clotted at room temperature. Serum-albumin was measured by direct spectrophotometry, using a colorimetric assay provided by Beckman (cat no. 46758). The serum total cholesterol was measured by means of an enzymatic colorimetric assay (Beckman; cat. no. 467825). Serum ferritin (Bayer; cat. no. 110746), -vitamin B12 (cobalamin Bayer; cat. no. 110748) and -folate (Bayer; cat.no. 118551) were all quantified by chem-iluminessence. Vitamin A (retinol) and vitamin E were quantified using High Performance Liquid Chromatography. Vitamin D was determined by means of a radioactive binding protein assay, during which proteins are removed from the sample, incubating the protein free sample with a salt of periodic acid, isolating 1,25-dihydroxy-vitamin D. The concentration of zinc in the plasma was measured using a commercial kit (Randox Laboratories Ltd., Crumlin, N. Ireland, UK; cat. no. ZN 2341) using a Hitachi analyser. Glutathione (GSH) levels were measured spectrophotonometrically by using a glutathione assay kit (Calbiochem, United Kingdom; cat. no. 35412Y). Blood concentrations of the population group were compared with local age-related normal values.

Ethical aspects

Written consent was obtained from the legal guardian of each child. The study was approved by the Ethics Committee of the Faculty of Health Sciences of the University of the Free State, South Africa.

Data entry and analysis

Frequencies and percentages were used to summarize the categorical data. Numerical data were summarised by means and quartiles due to skew distributions. Nonparametric statistical methods (Mann-Whitney and Kruskall-Wallis tests) were applied to compare within variables between subgroups. All analyses were done using SAS. Epi Info was used to calculate z-scores and percentiles of anthropometric data. Correlations were determined with the Spearman rank correlation test, with a p-value of less than 0.05 considered to be significant.

Results

General, growth and immune status

The median age (minimum; maximum) of the children was 5.35 years (1.16; 10.17), with 43% (n=16) female and 57% (n=21) male. The median (quartiles) values for the following markers were: WAZ -1.97 (-2.48; -1.4), HAZ -2.59 (-3.35; -2.03) and WHZ -0.66 (-0.85; 0.05). The median (quartiles) viral load (n=35) was 117 000 copies/ml (14 500; 305 000), with the median (quartiles) $CD4^+$ cell count 477 cells/mm^3 (300; 791) and $CD4^+$ percentage 22.5% (14.2; 35.8). According to the CDC Immunological classification, forty percent (14/35) had no evidence of immunosuppression (category 1), 34.3 % (12/35) were moderately suppressed (category 2) and 25.7 % (9/35) severely immunosuppressed (category 3).

Correlations between growth and immune status/disease progression

The median viral load and $CD4^+$ cell counts for the different HAZ categories are summarized in Table 5.1. Children categorized in the group with a HAZ of less than -3 SD (n=14) had a median viral load of 290 000 copies/ml versus 6560 copies/ml for the children in the group with a HAZ of more than -2 SD (n=7). This represented

a statistical difference approaching significance (p=0.06). No statistical significant differences could be demonstrated between $CD4^+$ cell counts or % in the different HAZ groups. No significant difference could be shown between $CD4^+$ cell counts or viral loads and WAZ or WHZ as well.

Table 5.1: Median viral load and $CD4^+$ cell count per height-for-age z-score category

Immune marker	HAZ < -3 SD (lower quartile; upper quartile)	HAZ -2 to -3 SD (lower quartile; upper quartile)	HAZ > -2 SD (lower quartile; upper quartile)	Difference (p-value: Kruskall-Wallis)
Viral load	290 000 (59 400; 489 000)	90 600 (14 500; 160 000)	6560 (411; 309 000)	0.06
$CD4^+$ cell count	442 (276; 736)	425 (305; 660)	592 (311; 1020)	0.74
CD4 %	22.7 (15.4; 25.1)	17.8 (12.3; 46.6)	31.2 (20.9; 51.1)	0.31

Spearman rank correlation coefficients indicated significant, but weak negative correlations between viral load and HAZ (r=-0.42; p=0.03) and WAZ (r =-0.35; p=0.04) respectively. No significant correlations could be demonstrated between growth indicators and $CD4^+$ cell counts or %.

Correlations between metabolic indicators, growth and immune status

As summarised in Table 5.2, children in the severely suppressed immune category had significantly lower (p=0.02) serum albumin levels, than children with no evidence of suppression. Non-significant declines in the median Hb and cholesterol from the Category 1 group to the Category 3 group could also be observed. No significant differences could be demonstrated between serum levels of folate, vitamin B12 and ferritin in the various immune categories.

According to Spearman correlation coefficients, significant but weak positive correlations were demonstrated between the age of the children and serum levels of folate (r=0.51) and albumin (r=0.42). Significant but weak positive correlations, were demonstrated between HAZ and s-albumin (r=0.44) as well. Serum levels of Hb (r= -0.58), albumin (r=-0.5), folate (r=-0.47), haematocrit (r=-0.46) and cholesterol (-0.35) showed significant, but moderate to weak, negative correlations with viral load.

Growth and serum levels of micronutrients/antioxidants

Low values of micronutrients were observed with reference to zinc (38.2%), vitamin A (62.5%), vitamin D (43.8%), vitamin E (12.5%) and glutathione (91.2%). According to Spearman correlation coefficients, a significant but weak positive correlation could be demonstrated between WHZ (r=0.42; p=0.03) and glutathione levels. A weak positive correlation approaching significance (r=0.31; p=0.06) was demonstrated between WAZ and serum levels of glutathione. Apart from glutathione, no significant correlations could be demonstrated between serum levels of vitamin A, D, E, zinc and the z-scores for weight-for-age, height-for-age and weight-for-height.

Table 5.2: Median viral load, metabolic indicators and micronutrient levels according to CD4% - Age-corrected CDC immunologic classification

Parameter (n; LQ; UQ)	Normal range	Median values	Category 1 No evidence of suppression	Category 2 Moderately suppressed	Category 3 Severely suppressed	Difference (p-value; Kruskall-Wallis)
Viral load copies/ml		**117000**	5630 (n=14; 400; 43 400)	205 000 (n=11; 102 000; 480 000	247 000 (n=9; 167 000; 294 000	0.001
Albumin g/l	**37 – 52**	**32**	34 (n=14; 33; 43)	31 (n=12; 29; 33)	28 (n=9; 25; 32)	0.02
Cholesterol mmol/l	**3 – 5**	**3**	3.15 (n=14; 2.8; 3.9)	2.9 (n=12; 2.6; 3.2)	2.8 (n=9; 2; 2.8)	0.09
Hemoglobin g/dl	**11.5 – 13.5**	**10.8**	11.6 (n=14; 10.5; 12.5)	10.4 (n=12; 9.7; 11.6)	9.8 (n=9; 8.7; 11,.8)	0.21
Ferritin ng/ml	**12 - 55**	**24**	22.5 (n=14; 18; 29)	25 (n=12; 9; 41.5)	25 (n=9; 22; 55)	0.45
Folate nmol/ml	**> 12.19**	**36.2**	44.6 (n=14; 36.1; 54.4)	29.2 (n=12; 25.1; 54.4)	33.1 (n=9; 29.7; 45.1)	0.22
Vitamin B12 pmol/ml	**156 - 672**	**483**	505 (n=14; 436; 581)	452 (n=12; 412; 636)	502 (n=9; 349; 674)	0.96
Vitamin A µg/dL	**20 - 43**	**18.15**	22.7 (n=13; 17.7; 24.8)	16.7 (n=11; 14.6; 20.8)	16.65 (n=8; 12.15; 18.65)	0.05
Vitamin D ng/ml	**20 - 60**	**20**	24 (n=13; 20; 26)	19 (n=11; 16; 21)	17 (n=8; 16; 22.5)	0.1
Vitamin E mg/dl	**3 – 9**	**8.05**	8.2 (n=13; 6.7; 9.6)	7.6 (n=11; 6.3; 10.3)	8.25 (n=8; 7; 9.05)	0.97
Zinc µmol/l	**12 - 17**	**12**	14.5 (n=14; 12; 15)	12 (n=12; 11; 12.5)	11 (n=8; 10.5; 11)	0.001
Glutathione mg/dl	**24 - 37**	**18**	19.5 (n=14; 17; 23)	17 (n=12; 11.5; 19.5)	17 (n=8; 13.5; 19.5)	0.12

Immune status and serum levels of micronutrients

Significant weak to moderate negative correlations, were shown between viral load and the following serum levels of antioxidants: vitamin A (r=-0.46), vitamin D (r=-0.39) and zinc (r=-0.36). Glutathione levels have shown a non-significant (p=0.06) weak negative (r=-0.33) correlation with viral load. Assessing the micronutrient levels between the different groups classified according to the CDC CD4% classification (Table 5.2), a significant difference could be demonstrated between group 1 (no suppression) and group 3 (severe suppression) in relation to levels of serum zinc ($p<0.01$) with vitamin A ($p=0.05$) approaching significance.

Children with abnormal low serum zinc levels (Table 5.3), had median CD4+ cell count of 300 cells/mm^3, compared to 736 cells/mm^3 in the group with normal serum zinc levels, reflecting a significant ($p=0.002$) difference. Although not significant ($p=0.06$), a similar tendency could be shown with the zinc deficient children having a median viral load of 250 500 (5.39 log) versus 43 400 (4.63 log) in the normal group. Children with abnormal low vitamin A levels, presented with significantly ($p=0.01$) higher viral loads and lower CD4+ cell counts ($p=0.05$ approaching significance) as well. Both of these indicators could have been influenced by the acute phase response and therefore, should be treated with caution. Despite similar trends in relation to glutathione, vitamin D and vitamin E (CD4 difference approaching significance), no significant differences could be demonstrated in relation to immune indicators.

Table 5.3: Comparison of immune status/disease progression between children with abnormal serum levels of micronutrients versus children with normal values

Variable	Immune	Median value in group with low levels	Median value in group with normal levels	p-value
Zinc	n CD4+ cell count Viral load	13 300 (229; 373) 250 500 (109 500; 372 000)	21 736 (463; 872) 43 400 (4700; 247 000)	 <0.01 0.06
Glutathione	n CD4+ cell count Viral load	31 463 (276; 791) 167 000 (6560; 309 000)	3 749 (530; 1240) 28 100 (14 500; 117 000)	 0.2 0.3
Vitamin D	n CD4+ cell count Viral load	14 407(300; 736) 210 000 (75 400; 309 000)	18 615 (276; 1020) 49 100 (4700; 305 000)	 0.34 0.18
Vitamin E	n CD4+ cell count Viral load	4 257 (201; 381) 360 500 (180 700; 466 500)	28 615 (323; 850) 117 000 (6560; 305 000)	 0.05 0.2
Vitamin A	n CD4+ cell count Viral load	20 362 (244; 726) 215 000 (90 600; 435 000)	12 770 (434; 1130) 21 300(400; 171 550)	 0.05 0.01

Table 5.4: Median (quartiles) values according to WHO clinical classification

Parameter	Median values for group (n=37)	Category 1 (n=4)	Category 2 (n=7)	Category 3 (n=7)	Category 4 (n=19)	Difference (p-value; Kruskall-Wallis)
Albumin g/l	**32**	35.5 (33; 41)	33 (31; 35)	34 (30; 38)	29 (25; 34)	0.06
Hb g/dl	**10.8**	12.3 (10.4; 13.5)	10.5 (10.1; 12.5)	11.8 (11.3; 11.9)	9.8 (8.8; 11.3)	0.08
Vitamin A µg/dL	**18.15**	18.45 (16.7; 25.2)	19.75 (15.6; 23.3)	19.5 (16.6; 24.3)	17.9 (12.25; 20.4)	0.47
Vitamin D ng/ml	**20**	21 (18; 29)	19.5 (16; 28)	23 (16; 26)	20 (16; 20)	0.62
Vitamin E mg/l	**8.05**	8.3 (7.8; 9.6)	9.25 (7.8; 9.9)	8 (7.6; 8.4)	7 (6.2; 10.2)	0.57
Zinc µg /l	**12**	11.5 (11; 13.5)	13 (11; 15)	12 (11; 14)	12 (11; 14.5)	0.95
Glutathione mg/dl	**18**	21.5 (19; 23.5)	20 (16; 23)	17 (13; 20)	17 (13.5; 19)	0.13

As summarised in Table 5.4, no significant differences could be demonstrated between serum levels of albumin, haemoglobin, vitamin A, D, E, zinc, glutathione and the stage of the disease. Although downward trends could be observed, especially in regard to s-albumin and haemoglobin, levels did not significantly decrease from Category 1 to 4.

Discussion

Pediatric HIV infection is associated with growth failure in most settings, even in children receiving antiretroviral drugs (McKinney *et al*., 1993). HIV-infected children in developing countries and specifically South Africa (Eley *et al*. 2002a), experience more severe growth retardation than observed in HIV-infected children in developed countries (McKinney *et al*., 1993).

Correlation between growth and immune status/disease progression

Arpadi *et al.* (2000) has demonstrated an inverse relationship between viral replication and growth velocity in a small group of HIV-infected children with growth failure. In this study, a similar trend could be observed. However, no significant differences could be demonstrated between growth indicators and median viral loads or $CD4^{+}$ cell counts, probably due to the small sample size. Spearman correlation coefficients have shown significant, but weak, negative correlations between viral load and WAZ as well as HAZ. These results are therefore in accordance with reported data from trials outside South Africa, indicating that children with high viral loads are more likely to be underweight and stunted. Similar correlations could not be demonstrated between $CD4^{+}$ cell counts or % and growth indicators in children in this sample. Stunted and underweight HIV-infected children in care centers are therefore likely to have higher viral loads.

Correlation between metabolic indicators, growth and immune status

Previous studies amongst HIV-infected children in South Africa indicated a significant relationship between immunological status and Hb levels (Eley *et al*. 2002b). In this study, children with higher viral loads had significantly lower Hb levels as well. A similar trend could be observed between CD4% and Hb levels where median the Hb value in the Category 3 group was lower than in the Category 1 group. There was no correlation between the prevalence of iron depletion and immune suppression in this particular sample. Serum ferritin levels could however be influenced by the acute-phase response and as c-reactive protein levels were not available, these results need to be treated with caution.

Decreased serum levels of albumin and cholesterol significantly correlated with higher viral loads, lower CD4 %, lower CD4:CD8, as well as stunting. Unlike HIV-infected children in developed countries where abnormal protein levels could not be demonstrated (Henderson *et al.*, 1997), the majority of children in this study not only had abnormal low values, but it was correlated with a compromised immune system as well. Stunted HIV-infected children in care centres, are therefore more likely to have anaemia and decreased serum levels of albumin and cholesterol.

Growth and serum levels of micronutrients

Despite widespread micronutrient deficiencies in this group, reflecting data from other South African (Eley *et al*., 2002a) studies, only glutathione levels correlated with growth indicators. These results therefore seem to be in accordance with a study by Arpadi *et al.* (1993) where the GSH levels in HIV-infected children were decreased by 24% if compared to HIV-negative controls. HIV-infected children with growth failure had GSH levels which were reduced by 35%.

There was no correlation between z-scores for weight-for-age, height-for-age and weight-for-height and serum levels of vitamin A, zinc, vitamin D or vitamin E.

Immune status and serum levels of micronutrients

In relation to the micronutrients, children with abnormal low vitamin A and zinc levels had significantly higher viral loads and lower $CD4^+$ cell counts. The micronutrient levels should be treated with caution, as biomarkers of acute phase response binding proteins were not available. Though no significant differences could be demonstrated in relation to vitamins D and E, children with abnormal low levels of these antioxidants had higher viral loads and lower $CD4^+$ cell counts. If indeed these deficiencies are related to immune status rather than growth, it remains to be seen if these deficiencies can be reversed by nutritional supplementation and whether it will impact on the immune status as well.

Summary

The limited sample size was one of the biggest limitations of this study. From more than 150 HIV-affected children screened, only 37 turned out to be HIV-infected, resulting in limited data. Another weakness was the failure to determine C-reactive protein levels to control for acute phase response.

With viral load measurements difficult to obtain in developing countries, growth velocities provide an informative index of disease severity and outcome. In the absence of available viral loads for HIV-infected children in care centres, the severity of stunting may be an indicator of disease progression. In situations where regular health screening is not available to HIV-infected children in care centres, height-for-

age may be an easy, valuable tool to identify children in need of further clinical examinations. Although growth correlated with serum levels of albumin and the presence of anaemia, apart from glutathione, no correlations were demonstrated between micronutrients and growth indicators. Children with micronutrient deficiencies tended to have higher viral loads and lower $CD4^+$ cell counts.

The prevalence of stunting in HIV-infected children in care centres is associated with a suppressed immune system as well as abnormal metabolic indicators. Micronutrient deficiencies are correlated with immune suppression as well. The results from this study underlines the importance that antiretroviral drugs and aggressive nutrition supplementation might play in restoring the immune, nutritional and health status in HIV-infected children.

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References

Arpadi SM, Cuff PA, Kotler DP, Wang J, Bamji M, Lange M, Pierson RN, Matthews DE (2000) Growth velocity, fat-free mass and energy intake are inversely related to viral load in HIV-infected children. *Journal of Nutrition* **130**, 2498 – 2502.

Arpadi S, Zang E, Back S, Muscat J, Ng S, Richie J (1993) Glutathione in HIV-infected children with growth failure. *International Conference of AIDS*. June 6 -11; **9,**309 (abstract no. PO-B05-1042).

Berhane R, Bagenda D, Marum L, Aceng E, Ndugwa C, Bosch RJ, Olness K (1997) Growth failure as a prognostic indicator of mortality in pediatric HIV infection. *Pediatrics* **100(1)**, E7.

Centre for Disease Control and Prevention (CDC) (1994) 1994 Revised Classification System for HIV Infection in Children less than 13 years of age. Official Authorized Agenda: Human Immunodeficiency Virus Infection Codes and Official Guidelines for Coding and Reporting ICD-9-CM. *Morbidity and Mortality Weekly Report* **43(RR-12)**, 2-3.

Cogill B (2003) *Anthropometric Indicators Measurement Guide. Food and Nutrition Technical Assistance*. 2003 edition. [Accessed 2004 August 15]. Available from: http:/www. Fantaproject.org/publications/anthropom.shtml

Eley B, Sive A, Kossew G, Abelse L, Cooper M, Hussey G (2002a) Growth and micronutrient disturbances in stable, HIV-infected children in Cape Town. *Annals of Tropical Paediatrics* **22**, 19 – 23.

Eley BS, Sive AA, Shuttleworth M and Hussey GD (2002b) A prospective, cross-sectional study of anaemia and peripheral iron status in antiretroviral naive, HIV-infected children in Cape Town, South Africa. *BMC Infectious Diseases* **2**,

Feldman JG, Burns DN, Gange SJ, Bachetti P, Cohen M, Anastos, K, Nowicki, M, Delapena, R, Paolo M (2000) Serum albumin as a predictor of survival in HIV-infected women in the Women's Interagency HIV Study. *Acquired Immune Deficiency Syndromes* **14**, 863 – 870.

Garg S & Miller TL (1999) Special considerations for the pediatric patient, in *Nutritional aspects of HIV infection* (eds TL Miller & SL Gorbach), pp. 109 – 134, Oxford University Press: New York.

Henderson RA, Talusan, K, Hutton, N, Yolken, RH, Caballero, B (1997) Serum and plasma markers of nutritional status in children infected with the human immunodeficiency virus. *Journal of the American Dietetic Association* **97(12)**, 1377 – 1381.

Irlam JH, Visser, ME, Rollins, N & Siegfried, N (2006) Micronutrient supplementation in children and adults with HIV infection (Review). The Cochrane Collaboration. *The Cochrane Library*, Issue 2, John Wiley & Sons, Ltd: Chichester.

Keen CL, Uriu-Adams JY, Ensunsa JL & Gerswin ME (2004) Trace Elements/Minerals and Immunity, in *Handbook of Nutrition and Immunity* (eds ME Gershwin, P Nestel, & CL Keen), pp. 117 – 140, Humana Press: New Jersey.

Mastroiacovo P, Ajassa C, Berardelli G, Bruni R, Catania N, Fidanza A, Pace V & Zanzoglous S (1996) Antioxidant vitamins and immunodeficiency. *International Journal for Vitamin and Nutrition Research* **66(2)**, 141.

McKinney RE, Robertson WR and the Duke Pediatric AIDS Clinical Trials Unit (1993) Effect of human immunodeficiency virus infection on the growth of young children. *Journal of Pediatrics* **123**, 579 – 582.

Pollack H, Glasberg H, Lee H, Nirenberg A, David R, Krasinski K, Borkowsky W & Oberfield S (1997) Impaired early growth of infants perinatally infected with human immunodeficiency virus: correlation with viral load. *Journal of Paediatrics* **130**, 915 – 922.

Semba RD (1997) Vitamin A and human immunodeficiency virus infection. *Proceedings of the Nutrition Society* **56**, 459 – 469.

Tang AM, Lanzillotti J, Hendricks K, Gerrior J, Ghosh M, Woods M & Wanke C (2005) Micronutrients: current issues for HIV care providers*. Acquired Immune Deficiency Syndromes* **19**, 847-861.

Tomkins AM (2005) Evidence-based nutrition interventions for the control of HIV/AIDS. *South African Journal of Clinical Nutrition* **18(2)**, 187 – 191.

Visser, M (2005) Nutritional Prophylaxis, in *HIV/AIDS in South Africa* (eds SSA Karim & QA Karim), pp. 463 – 467, Cambridge University Press: Cambridge.

CHAPTER 6

IMPACT OF NUTRITION INTERVENTION WITH AN ENZYME MODIFIED MAIZE/SOY BLEND ON THE GROWTH, IMMUNE, MICRONUTRIENT AND HEALTH STATUS OF HIV-INFECTED CHILDREN IN CARE CENTRES, IN MANGAUNG – A RANDOMISED TRIAL

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Abstract

Objective: To describe changes in growth, immune, micronutrient and health status in food secure HIV-infected children after nutrition intervention with modified maize/soy blends.

Design: Double blind, placebo controlled, prospective clinical trial.

Methods: A nutrition intervention study of 120 days was undertaken in 37 antiretroviral naïve, HIV-infected children in care centres in Mangaung. Children were randomised into two groups, according to CD4 concentrations and weight-for-age z-scores. The first group (n=19) received an experimental product (E) with an additional enzyme mixture; the other (n=18) a placebo (P). Non-parametric methods were used for statistical analysis.

Results: Median changes included non-significant decreases in median viral loads of 30 145 copies/ml for E and 350 copies/ml for P. The median (quartiles) $CD4^+$ cell count increases were 38.5 (-17; 180) cells/mm^3 for E (p=0.07) and 167 (-160; 575) cells/mm^3 for P (p=0.76). No significant differences relating to growth and changes in micronutrient levels could be demonstrated between the two groups. Children in P experienced a significant (p=0.003) decrease in serum albumin levels, while the E group maintained their levels, resulting in a significant difference.

Conclusion: Nutrition supplementation in both groups led to non-significant improvements in median viral loads and $CD4^+$ cell counts. There was a significant difference between the two groups in relation to changes in serum albumin levels. None of the groups benefited in terms of growth, health status or micronutrient status, despite good compliance resulting in micronutrient intakes in excess of 100% RDA.

Introduction

Growth failure in children is an important manifestation of HIV-infection and has been shown to be an independent risk factor of death.[1] Progressive stunting appeared to be more typical than wasting and a number of studies in developed countries, demonstrated an inverse relationship between height growth velocity and viral load.[2] In developing countries, growth failure may be a bigger problem than in developed countries as has been demonstrated by Eley et al.,[3] with as many as 55 to 60% of HIV-infected children stunted and 25 to 30% underweight.

Micronutrient deficiencies can also adversely affect growth[4] and seem to affect more than 60 percent of HIV-infected children in South Africa.[3] Although micronutrient interventions in HIV-infected adult populations have been widely investigated,[5] only a small number of studies have been done on HIV-infected children. [6,7]

The largest number of completed micronutrient studies, to date, in HIV-infected children have investigated vitamin A deficiency. Unlike study results in HIV-infected adults where vitamin A supplementation does not seem to positively affect disease outcome,[6] studies in children indicate that periodic supplementation with vitamin A is associated with a reduction in all-cause mortality and AIDS-related deaths.[8] Outcomes from a zinc supplementation trial, indicated that 10 mg of zinc per day, could reduce morbidity caused by diarrhoea and did not result in an increase in viral load in HIV-infected children.[9] Micronutrient supplementation without macronutrient supplemention is, however, not going to reverse underweight and stunting. To increase energy intake amongst HIV-infected children in developed countries, several options in the form of enteral or parenteral nutrition are available. Nutrition support with gastrostomy feeding

tubes,[10,11] resulted in improved body weight, especially in children with higher initial $CD4^+$ cell counts. The data strongly suggest that nutritional interventions that are provided early, are affordable and available may have a dramatic impact on the course of HIV disease in children.

According to Morgan et al.,[12] progression rates to AIDS in Africa are similar to those in developed countries; however, rates of all-cause mortality are much higher and progression times to death are shorter than in developed countries. Nutritional care and support may therefore be of more benefit to children living with HIV/AIDS in developing countries, because underlying factors contribute to a poor baseline nutritional status which impacts on disease progression and outcome.[13] Nutrition support in the form of enteral or parenteral support in developing countries is, however, nor accessible, nor affordable for the majority of children living with HIV/AIDS.

The goals of nutrition support in these cases should be to increase the amount and frequency of food intake. Although food-based approaches should always be the intervention of choice, HIV-infected individuals have macro and micronutrient needs that far exceed those of HIV-negative individuals, and supplements may make it easier to reach those goals.[14] The most common form of supplemental feeds used in developing countries to increase energy and nutrient intake, include blended maize/soy flour, food parcels or ready-to-use food supplements (RTUF). To increase the energy and nutrient density of foods, protein and other micronutrients should be added or amylase can be added to reduce the viscosity (thickness) of solid foods.[15]

Instant enriched soy/maize blends have been in use for a number of years in most of the provinces in South Africa as part of the health facility based program (previous protein-energy-malnutrition scheme). Similar supplements used in other parts of Africa, have come under scrutiny due to low bio-availability of iron and zinc, as a result of high concentrations of phytic acid. Strategies that have been used to enhance mineral bioavailability in cereal-based complementary foods, include enzymatic or non-enzymatic hydrolysis of phytic acid (hexa-inositol phosphate) and penta-inositol phosphate that do not inhibit zinc or iron absorption. The bioavailability of iron and zinc can also be improved by promoting the intake of enhancers, for example ascorbic acid.[16] Another option is to partly remove the water soluble phytates by either soaking or by the hydrolysis of the phytates, thereby increasing the absorption of zinc and iron. By further fortifying the staple with other micronutrients, most of the intrinsic deficiencies can be overcome.[14]

The presence of infection (eg. HIV/TB) may also cause loss of appetite, which can lead to a decrease in food- and energy intake. The total solids content of a diet based on starch containing foods may be limited by the resulting thickness or viscosity of the cooked product. The viscosity of such a mix can be reduced, without sacrificing its energy density, by adding an exogenous source of amylase to liquefy the gelatinised starch. Several sources[17,18] indicate that the addition of amylase to a starch based diet can significantly increase energy intake in children.

Findings as summarised by Long and Santos,[19] indicate that micronutrient supplementation can be used as an important tool to reduce morbidity and mortality in children in developing countries. Recent reports that provide detail on improvements in clinical status or quantitative evaluations of the impact of the interventions, are

lacking.[20] It is therefore difficult to draw any conclusions as to the efficacy of these interventions on disease progression in HIV-infected children.

In this particular study, the researchers proposed to investigate the impact of nutrition supplementation with fortified maize/soy blends on the growth, immune, micronutrient and health status in antiretroviral naïve, clinically stable HIV-infected children in care centers in Mangaung.

Methods

Study design and sampling

One hundred and fifty five food-secure HIV-affected children in care centres in Mangaung, in the central part of South Africa, were screened to determine HIV status. A randomised, double blind, clinically controlled, prospective trial was subsequently undertaken on 37 clinically stable, antiretroviral naïve, HIV-infected children. Children with a positive enzyme-linked immunosorbent assay (ELISA), were purposely sampled and recruited into the study between August 2004 and March 2005. Children with acute illness or episodes of hospitalisation in the preceding week were excluded from the study. All the children received co-trimoxazole prophylaxis and were dewormed prior to the intervention.

Growth

Growth was determined by the median z-score differences in weight-for-age from baseline to the end of the intervention, as well as the weight growth velocity (g/kgday). Anthropometric measurements were done by a trained dietitian using standardised techniques.[21] These measurements were done at baseline and at monthly intervals until the end of the study. A SECA electronic scale (model 708) was used to determine

the weight of the lightly clothed children to the nearest 0.1 kg. A stadiometer was used to obtain the height of the children older than 24 months to the nearest 0.1 centimeter. In children younger than 24 months, the recumbent length was measured. The weight and height were compared with the norms for physical growth using the NCHS standards.

Immune status and biochemistry

Fasting blood samples were collected by a trained medical doctor. Samples were taken from the antecubital vein after the puncture site was cleaned with alcohol. Two 5 ml EDTA samples (Vacutainer) were wrapped in foil to protect it from light and stored on ice immediately after collection. For the purpose of this study, immune status referred to the viral load and CD4 concentrations. The plasma viral load (log_{10} HIV-1 RNA copies/ml) was quantified by means of a nucleic acid amplification test on a COBAS AMPLICOR (Roche Diagnostic System Inc.). The $CD4^+$ lymphocyte cell count was determined on EDTA-anti-coagulated whole blood samples using a flow cytometer (BD Biosciences FACSCalibur). The $CD4^+$ counts and percentages were used to classify children into immunological categories according to the Centre for Disease Control (CDC) guidelines.

Plasma chemistry concentrations were quantified using serum prepared from whole blood, clotted at room temperature. Serum albumin was measured by direct spectrophotometry, using a colorimetric assay. The serum total cholesterol was measured by means of an enzymatic colorimetric assay provided by Beckman (cat no. 46758). Serum ferritin (Bayer; cat. no. 467825), vitamin B12 (cobalamin; Bayer; cat. no. 110748) and folate (Bayer; cat. no. 118551) were all quantified by chem – iluminessence. Vitamin A (retinol) and vitamin E were quantified by using High

Performance Liquid Chromatography. Vitamin D was determined by means of a radioactive binding protein assay, during which proteins are removed from the sample, incubating the protein free sample with a salt of periodic acid, isolating 1,25 dihydroxy-vitamin D. The concentration of zinc in the plasma was measured using a commercial kit (Randox Laboratories Ltd., Crumlin, N. Ireland, UK; cat. no. ZN 2341) using a Hitachi analyser. Glutathione (GSH) levels were measured spectrophotometically by using a glutathione assay kit (Calbiochem, United \kingdom; cat.no. 35412Y). Blood concentrations of the population group were compared with local age-related normal values. Values between baseline and end data were compared to determine changes over time.

Clinical features

Health status was determined by means of a clinical examination undertaken by a trained medical doctor. The clinical examination included an assessment of the child's vital signs e.g. temperature, pulse rate, respiratory rate and an examination of all the systems: general, respiratory, cardiovascular, abdominal and neurological. Children were categorised according to the WHO Clinical Staging of HIV for Infants and Children with established HIV Infection. [22] Clinical features were assessed at baseline, followed by monthly intervals until the end of the study.

Intervention

After collection of the baseline data, the sample was stratified according to weight-for-age z-score, $CD4^+$ count and/or CD4 percentage, presence of TB and the place of residence. The children were then randomly placed in either the Experimental (E) group (n=19) or the Placebo (P) group (n=18), by the Department of Biostatistics, Faculty of Health Sciences, University of the Free State. The researchers were blind to

the randomisation process as well as to the products the patients received. Children in both groups were supplemented with 150g (dry weight) of the experimental product (enzyme-modified instant enriched maize/soy blend) or placebo (instant enriched maize/soy blend) on a daily basis, for four days per week (Table 6.1). This resulted in an additional 1800 kJ/day, apart from their normal diet in the care centre. As a number of the children were discharged over week-ends to the extended family and one of the centres only had funds to operate four days per week, the decision was made to supplement the diet for only four days every week. The supplements were provided as breakfast replacement to the children. Both products were identical in macro and micronutrient content, except for the added enzyme-mixture (containing amylase, protease, ascorbic acid, organic sulfur and n-acetyl-cysteine) in the experimental product. Monitoring was done in order to ensure that the applicable children receive the correct supplements and quantities. Results from plate wasting, indicated that left-over supplements were weighed 55 to 63 (minimum; maximum) times, out of a potential 120 times per child, during the intervention period. A median of less than 10 g (wet weight) of the supplement were left in the plate, indicating that the children consumed most of the 150g dry (450 g wet) portion provided, which indicated good compliance.

Until after collection of the end values, only the manufacturer knew which of the two products contained the enzymes. The vitamin/mineral mixtures for both the test product and the standard enriched mealiemeal was bought as a pre-mix from Roche Laboratories and added to the soy-maize blend at the factory. Two markers in both products were analysed by an independent laboratory (SABS analysis) to ensure that micronutrient values corresponded to the specification.

The experimental product and placebo contained the following macro- and micronutrients:

Table 6.1: Nutritional analysis of experimental product and placebo

Nutritional content	*Amount per 100 g serving size*	
	Experimental	*Placebo*
Protein (g)	14.0	14.0
Total Carbohydrate (g)	61.2	61.2
Fat (g)	14.8	14.8
Energy (kJ)	1765.0	1765
Isoflavones (mg)	94	94
Vitamin A (mcg RE)	600	600
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 B 12 (mcg)	2.5	2.5
Biotin (mcg)	50	50
Pantothenic acid (mg)	4	4
Vitamin K (mcg)	30	30
Sulfur (mg)	**34**	**0**
Calcium (mg)	825	825
Phosphorus (mg)	660	660
Potassium (mg)	550	550
Iron (mg)	10	10
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 cysteine (mg)	**120**	**0**
Amylase	**+**	**-**

Ethical aspects

Written consent were obtained from the care centres as well as the legal guardian of each child. The study was approved by the Ethics Committee of the Faculty of Health Sciences of the University of the Free State, South Africa.

Data entry and analysis

The statistical analysis was done by the Department of Biostatistics at the University of the Free State. Data was processed using SAS. Descriptive statistics including frequency distribution was used to summarise the categorical data. The distribution of most of the numerical variables was skewed. It was therefore decided to use the percentiles to summarise numerical variables. Nonparametric statistical methods (Mann-Whitney and Kruskall-Wallis tests; Spearman correlation coefficients) were used to compare within variables between subgroups. Epi Info was used to calculate z-scores and percentiles of anthropometric data. Health, immune, biochemical and growth parameters after 16 weeks into the study, were compared to baseline values in order to determine whether any significant changes took place, using 95 % confidence intervals.

Results

General

From the sample of 37 HIV-infected children included in the study, only 29 completed the intervention (15 in P; 14 in E). Eight children dropped out (2 deceased; 1 hospitalized; 1 refused the supplement; 2 with chronic diarrhea; 1 adopted; 1 only attended day care intermittently).

The median age (minimum; maximum) of the children in group E and P were 5.5 years (2.3; 9.8) and 5.2 years (2.4; 10.2) respectively. The median (quartiles) values for the weight-for-age z-score for group E was -1.77 SD (-2.39; -1.06) and for group P -1.97 SD (-2.95; -1.45). The median (quartiles) baseline viral load for group E was 83050 copies/ml (6560; 286 000) and for group P 102 000 copies/ml (14 500;

294 0000). The median (quartiles) $CD4^+$ count and percentage for group E were 503 cells/mm^3 (351; 744) and 22.62 % (19.56; 34.4) respectively. The median (quartiles) $CD4^+$ count and percentage for group P were 557 cells/mm^3 (276; 791) and 23.18 % (12.34; 38.36) respectively. There were no significant differences between the baseline growth, immune, micronutrient and health status indicators between the two groups (Mann-Whitney test) at baseline.

Outcome on growth

Despite 66% (n=10) of children in group P and 79% (n=11) of children in group E gaining weight, no significant catch-up growth could be demonstrated, with median changes in the weight-for-age z-scores +0.04 (-0.26; 0.31) SD for group P and -0.06 (-0.16; 0.18) SD for group E respectively. Median growth velocities (quartiles) of only 0.38 g/kg/day (-0.07; 0.77) for the placebo group and 0.31 g/kg/day (0.04; 0.47) for the experimental group has been achieved.

Outcome on changes within immune markers

As summarised in Table 6.2, children in group E experienced a median viral load decrease of 30 147 RNA copies/ml, with the median decrease in group P, 350 copies/ml. There was a non significant (p=0.07) increase in the median $CD4^+$ cell count of group E of 38.5 cells/mm^3 as well as an increase (p=0.4) of 68 cells/mm^3 in group P. There were no significant differences between improvements in group E and P. Less than half of the total group showed viral load reductions of more than 0.5 log, which is of any statistical significance. Only one child in this sample had a viral load reduction of more than 1 log, indicating clinical significance, and only two children improved to become virus undetectable.

Table 6.2: Comparison of median changes in and between the two groups after intervention

Variable	Normal reference range	Experimental Group (n=14): Change in group	P-value Difference in group (Signed rank test)	Placebo (n=15): Change in group	P-value Difference in group (Signed rank test)	P-value Difference between groups (Mann-Whitney test)
Viral load (copies/ml)		- 30 147 (-190000; -350)	0.15	-350 (-176 856; + 35 980)	0.3	0.63
CD4 cell count cells/mm^3		+ 38.5 (-17; +180)	0.07	+68 (-114; +163)	0.76	0.4
CD8 cell count cells/mm^3		+ 164.5 (-171; + 417)	0.3	+167 (-160; +575)	0.14	0.69
CD4 %		+0.39 (-1.29; +3.2)	0.39	-1.61 (-4.8; +2.5)	0.23	0.15
Vitamin A µg/dl	20 - 43	-1.9 (-3.9; -0.1)	0.11	-0.3(-5.2; 2.3)	0.6	0.63
Vitamin E mg/dl	3 - 9	-0.2(-0.7; 0.9)	0.86	-0.6(-1.9; 0.2)	0.17	0.31
Vitamin D ng/ml	20 - 60	0(-2; 2)	0.1	-1(-5; 4)	0.96	0.62
Zinc µmol/l	12 - 17	0(-1; 1)	1	0(-1;0)	0.51	0.6
Glutathione mg/dl	24 - 37	0.5(-1; 3)	0.66	4(0;10)	0.02*	0.05
S-albumin g/l	37 - 52	+1 (-1; 2)	0.29	-2(-3; 0)	0.003*	0.01*
S-ferritin ng/ml	12 - 55	+3.5 (3; 13)	0.03*	+8 (-2; 22)	0.06	0.7
Folate nmol/l	> 12.19	+0.1 (-2.5; 6.5)	0.3	+0.3(-1.4; 9.7)	0.38	0.8
Vitamin B12 pmol/l	156 - 672	+209.4 (-10.4; +475.5)	0.01*	+167(+70.2; +269.3)	<0.01*	0.86
Haemoglobin g/dL	11.5 – 13.5	-0.15(-0.4; 0.4)	0.66	-0.2(-0.8; 0.1)	0.17	0.67

* Significant to the nearest 0.05 Confidence Interval

Outcome on changes within micronutrients

No significant differences could be demonstrated within or between both groups in terms of the following micronutrient levels: vitamin A, vitamin E, vitamin D and zinc. Glutathione levels increased significantly in group P, but apart from a significant (p=0.03) moderate positive (r=0.53) correlation with differences in the serum levels of zinc, no other explanation for this finding, including changes in growth parameters,

could be found. Serum levels of vitamin B12 showed significant increases in both the E and P groups.

Outcome on changes within health status indicators

Serum albumin levels decreased significantly (p=0.003) in the P group, despite no decrease in somatic growth indicators. This resulted in a significant difference (p=0.01) between the E and P groups in relation to serum levels of albumin after the intervention. Serum levels of ferritin showed a significant increase in the E group (p=0.03) and an increase approaching significance (p=0.06) in the P group. Biomarkers of inflammatory response were, however, not available to control for the presence of infections. There were no significant differences within and between groups regarding any clinical features monitored during the intervention.

Discussion

Impact of supplementation on growth

In a community based study on HIV-negative children supplemented with a maize/soy blend in Malawi, catch-up growth of 3.1 g/kg/day was observed over an intervention period of six months.[23] However, in this study, within HIV-infected children, supplementation with neither of the maize/soy blends resulted in significant improvements in growth.

Aggressive nutritional supplementation by means of gastrostomy tube feedings in a sample of 23 HIV infected children by Miller et al.,[10] showed no improvement in $CD4^+$ cell counts after a 6 month intervention period. Significant improvements in the weight-for-age Z-scores were, however, demonstrated. In order to facilitate improvements in weight in that particular study, the median energy intake was increased from

110kcal/kg/day (462 kJ/kg/day) before enteral supplementation to 189kcal/kg/day (794 kJ/kg/day) during gastrostomy tube feedings. These interventions are not available to and affordable for nutrition management of HIV-infected children in developing countries.

Children in this study had access to regular, balanced meals, further improved by replacing ordinary maize porridge for breakfast with a energy dense maize/soy blend, providing in excess of 100% RDA for micronutrients. Despite this intervention, children did not consume more than 400 – 500 kJ/kg/day (as determined by nutrition analysis of menus and supplements). This is clearly not sufficient to establish catch-up growth in antiretroviral naïve, HIV-infected children with growth failure. Current initiatives providing supplementation with only soy/maize blends of equal or less amounts of macro- and micronutrients, are not going to achieve significant results in terms of growth in HIV-infected children. In this study, no significant difference in terms of growth could be demonstrated between children consuming the enzyme-modified maize/soy blend versus the unmodified supplement. Additional sources of energy dense supplements such as RTUF and energy drinks should probably be added to current supplementation regimens, to achieve optimal results.

Impact of supplementation on the immune status

A general improvement in the immune status of children supplemented with both maize/soy blends, could be observed from changes in viral loads and $CD4^+$ cell counts, unlike the study by Miller et al.,[10] where children experienced no improvements in $CD4^+$ cell counts. Although children in the group consuming the enzyme-modified maize/soy blend demonstrated a median $CD4^+$ cell count increase approaching significance, significantly better results against the unmodified supplement could not

be demonstrated. Log reductions in viral load, which is seen to be clinically significant,[24] could not be achieved by means of nutrition support alone. It is therefore clear that the nutrition intervention strategies currently available to HIV-infected children in the largest part of South Africa, are inadequate to achieve significant improvements in immune status and that intervention with conventional therapies such as ARVs should be made available to all children, including AIDS orphans in care centres. Nutrition supplementation, however, had a general positive effect on the immune status of the majority of the children and should form part of optimal management of children living with HIV/AIDS.

Impact of supplementation on micronutrient levels

Despite supplementation of micronutrients in excess of RDA levels, no significant improvements in serum levels of vitamin A, D, E and zinc could be demonstrated. It is important to remember that serum levels of vitamin A and zinc could be reduced by the acute phase response and that serum levels therefore are not an accurate indication of true vitamin A and zinc status. However, no improvements in serum levels of vitamin D and E could be demonstrated as well, even in those children presenting with abnormal low levels at baseline. Multiple micronutrient supplementation at 100 to 150% RDA as provided to children in this study, therefore, did not significantly change serum levels of micronutrients in HIV-infected children. Only glutathione levels improved significantly in children in the placebo group, and this finding correlated with improved serum levels of zinc.

Impact of supplementation on health status

Nutrition supplementation had no impact on clinical features of children with HIV. Nutrition supplementation of two children had to be discontinued as a result of chronic

diarrhoea. The maize/soy blends containing 100% RDA of zinc did not improve the severity of the diarrhoea and the children had to be excluded from the study.

Children consuming the enzyme-modified product were able to maintain serum levels of albumin, also shown to be an important indicator of disease outcome.[25] This significant better outcome than with the children consuming the unmodified maize/soy blend, may be as a result from the protease in the enzyme mixture, aiding digestion and absorption of protein in the product. As both the experimental product and the placebo contained 14 g of protein per 100 g serving, children received on average 1g protein/kg bodyweight for breakfast which was a substantial amount of the daily protein intake. Further investigation should be done to determine the availability of protein in unmodified maize/soy blends.

Summary

The limited sample size was one of the biggest limitations of this study. From more than 150 HIV-affected children screened, only 37 turned out to be HIV-infected, resulting in limited data. Another weakness was the failure to determine c-reactive protein levels to control for acute phase response.

According to Visser,[13] increases in body weight and growth are linked to the introduction of antiretroviral therapy in children. The study did therefore contribute to underline the importance of the availability of antiretroviral drugs to all HIV-infected children and to increase the macronutrients currently available to HIV-infected children, receiving support from governmental nutrition regimens. Such changes are, however, dependent on the availability of sufficient funding within the Nutrition Directorates and can only be successfully implemented with the backing of decision makers at national level. As has been demonstrated in this study and underlined by

Guarino et al.,[26] nutritional rehabilitation may increase naïve $CD4^+$ cell counts, thereby allowing partial functional recovery of the immune response in children with HIV-infection.

More aggressive nutrition supplementation than soy maize blends alone, should be investigated to achieve rehabilitation of weight in HIV-infected children. Management of HIV-infected children in care centres, should be optimised by proper management with antiretroviral drugs as well.

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Disclosure

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References

1. Berhane R, Bagenda D, Marum L, Aceng E, Ndugwa C, Bosch RJ, Olness K. Growth failure as a prognostic indicator of mortality in pediatric HIV infection. Pediatrics. 1997; 100(1): E7.

2. Arpadi SM, Cuff PA, Kotler DP, Wang J, Bamji M, Lange M, Pierson RN, Matthews DE. Growth velocity, fat-free mass and energy intake are inversely related to viral load in HIV-infected children. J Nutr. 2000; 130: 2498 – 2502.

3. Eley B, Sive A, Kossew G, Abelse L, Cooper M, Hussey G. Growth and micronutrient disturbances in stable, HIV-infected children in Cape Town. Ann Trop Paediatr. 2002; 22: 19 – 23.

4. Arpadi SM. Growth failure in HIV-infected children. Consultation on Nutrition and HIV/AIDS in Africa: Evidence, lessons and recommendations for action, World Health Organization, Durban, 2005.

5. Tang AM, Lanzillotti J, Hendricks K, Gerrior J, Ghosh M, Woods M and Wanke C. Micronutrients: current issues for HIV care providers. AIDS. 2005; 19:847-861.

6. Irlam JH, Visser ME, Rollins N, Siegfried N. Micronutrient supplementation in children and adults with HIV infection (Review). The Cochrane Collaboration, Wiley Publishers, 2006.

7. Tomkins AM. Evidence-based nutrition interventions for the control of HIV/AIDS. SAJCN. 2005; 18(2): 187 – 191.

8. Fawzi W, Mbise R, Hertzmark E, Fataki M, Herrera M, Ndossi G and Spiegelman D. A randomized trial of vitamin A supplements in relation to mortality among HIV-infected and uninfected children in Tanzania, Pediatr Infect Dis J. 1999; 8: 127-133.

9. Bobat R, Coovadia H, Stephan C, Naidoo KL, McKerrow N, Black RE and Moss WJ. Safety and efficacy of zinc supplementation for children with HIV-1 infection in South Africa: a randomized double-blind placebo controlled trial. Lancet. 2006; 367: 814 – 815.

10. Miller TL, Awnetwant EL, Evans S, Morris VM, Vasquez IM and McIntyre K. Gastrostomy tube supplementation for HIV-infected children. Pediatrics. 1995; 4: 696 – 702.

11. Henderson RA, Saavedr, JM, Perman JA, Hutton N, Livingston RA and Yolken RH. Effect of Enteral Tube Feeding on Growth of Children with Symptomatic Human

Immunodeficiency Virus Infection. J Pediatr Gastroenterol Nutr. 1994; 18(4): 429 – 434.

12. Morgan D, Mahe C, Mayanja B, Okongo JM, Lubega R and Whitworth JA. HIV-1 infection in rural Africa: is there a difference in median time to AIDS and survival compared to indutrialized countries? AIDS. 2002; 16: 597 – 603.

13. Visser M. Nutritional Prophylaxis. In: Karim SSA and Karim QA, editors. HIV/AIDS in South Africa. Cambridge: Cambridge University Press, 2005; pp. 463 - 476.

14. Friis H, Gomo E and Michaelsen K. Micronutrient Interventions and the HIV Pandemic. In Friis H, editor. Micronutrients in HIV Infection. London: CRC Press, 2002; pp. 219 – 245.

15. Friis H. Micronutrients and Infections: An introduction. In Friis H, editor. Micronutrients in HIV Infection. London: CRC Press, 2002; pp. 1 – 21.

16. Gibson RS, Ferguson EL and Lehrfeld J. Complementary foods for infant feeding in developing countries. Eur J Clin Nutr. 1998; 52: 764 – 770.

17. Chinnamma J and Gopaldas T. 1993. Evaluation of the impact on growth of a controlled 6-month feeding trial on children fed a complementary feed of a high energy-low bulk gruel versus a high energy - high bulk gruel in addition to their habitual home diet. J Trop Paediatr. 1993; 39: 16 -22.

18. Den Besten L, Glatthaar II and Ijsselmuiden CB. 1998. Adding α-amylase to Weaning Food to Increase Dietary Intake in Children. A Randomised Controlled Trial. J Trop Paediatr. 1998; 44: 4 – 6.

19. Long KZ and Santos JI. Vitamins and the regulation of the immune response. Pediatr Infect Dis J. 1999, vol. 18: 283 – 290.

20. Willumsen, J. The role of nutrition interventions in the prevention of HIV infection and progression of HIV/AIDS. Working paper for World Health Organisation. May 2003. Geneva.

21. Cogill B. Anthropometric Indicators Measurement Guide. Food and Nutrition Technical Assistance. 2003 edition, pp. 17 - 38. Available from: http:/www. Fantaproject.org/publications/anthropom.shtml, [Accessed 2004 August 15].

22. WHO, Antiretroviral therapy of HIV infection in infants and children in resource-limited settings: towards universal access – Recommendations for a public health approach, World Health Organization, 2006, Geneva, Switzerland.

23. Manary MJ, Ndkeha MJ, Ashorn P, Maleta K and Briend A. Home based therapy for severe malnutrition with ready-to-use food. Archiv Dis Child. 2004; 89: 557 – 561.

24. Martin D. Appropriate Laboratory Monitoring of HIV. S Afr Med J. 2000; 90(1): 33 – 35.

25. Henderson RA, Talusan K, Hutton N, Yolken RH and Caballero B. Serum and plasma markers of nutritional status in children infected with the human immunodeficiency virus. JADA. 1997; 97: 1377 – 1381.

26. Guarino A, Spagnuolo MI, Giacomet V, Canani RB, Bruzzese E, Giaquinto C, Roggero P, Plebani A and Gattinara GC. Effects of Nutritional Rehabilitation on Intestinal Function and on CD4 Cell Number in Children with HIV. J Pediatr Gastroenterol Nutr. 2002; 34: 366 – 371.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Introduction

Multiple micronutrient supplementation is a common strategy included in supplementation regimens by the Department of Health in South Africa. Apart from macronutrients, all supplements (including maize/soy blends) currently provided as part of the health facility based programme, contain between 30 and 100 percent RDA of all micronutrients for children in the various age groups, per daily serving. Subclinical deficiencies of multiple micronutrients, rather than single micronutrient deficiencies, are likely to be prevalent in developing countries where access to high quality food is limited. Results from a few intervention trials using multiple micronutrient supplementation amongst young children, have not been encouraging and more research is needed to determine efficacy of such supplements (Ramakrishnan et al., 2004, pp. 109 - 110). No previous studies have been done in Southern Africa on the impact of nutrition intervention with fortified maize/soy blends on the growth, immune status, micronutrients associated with immune status and health status in vulnerable HIV-infected children.

The prevalence of HIV infection amongst children screened in care centres in Mangaung in this study was only 24 percent; a lot less than originally assumed by the researchers. However, no baseline information, prior to this study, was available in relation to growth, immune and health status in HV-infected children in care centres. Therefore, despite the small sample size, valuable information regarding the various indicators have been gathered. It was also possible to determine correlations between growth, immune and health status. A randomised, double blind, placebo controlled clinical trial was subsequently done on the 37 HIV-infected children. Differences in growth, immune and health status indicators

between baseline and end values were assessed to determine the impact of nutrition supplementation with maize/soy blends.

7.2 Limitations and validity

Some of the difficulties encountered with observational studies in terms of micronutrient intake and levels are overcome in intervention studies. However, the fact that micronutrient deficiencies usually co-exist and often interact with one another makes the design of these studies and interpretation of results very difficult. It therefore does not make sense to evaluate a single micronutrient in the context of multiple co-existing and interacting deficiencies (Friis & Michaelsen, 1998).

In this study an attempt was made to overcome some of the intrinsic weaknesses of nutrition intervention studies by controlling for the following:

- This study was in the form of a prospective, double-blind, randomised trial.
- Only the manufacturer knew which of the two products were the experimental or the placebo.
- Standardisation of anthropometry and calibration of measuring equipment were done on a weekly basis.
- Completion of the questionnaires was done by trained personnel/fieldworkers.
- To ensure that children in the experimental and placebo groups receive the correct supplements respectively, colour coding was used. The two products were packed in plastic bags of two different colours, mixed in two bowls of the same different colours and dished up in cups or bowls of the same colours.
- Platewasting was done by a fieldworker to ensure compliance in terms of intake of the supplement. Left overs were weighed more than 50% of the times the supplement was served to the children. Less than a median of 10 g of wet product

was left on the plates, indicating good compliance in terms of intake. Only one child refused the supplement on a consistent basis and was thus excluded from the study.

Problems experienced which could have influenced some of the post-intervention results were addressed as follows:

7.2.1 Accuracy of markers used to determine immune and micronutrient status

- Although absolute $CD4^+$ cell counts and viral load are the markers of choice to reflect immune status and disease progression in children, as with adults, it is important to remember that $CD4^+$ cell counts are physiologically higher in young children compared with adults. There is also a greater difference between and within individual variability (Cant et al., 2003, p. 1301). As CD4 percentages vary less with age (Martin, 2000), the CD4 percentage as well as CD4: CD8 were included as markers as well. As no better markers to measure immune status in children are currently available, by including both the viral load and CD4 concentrations, the aim of the study could still be achieved.

- C-reactive protein measurements were not included in the study protocol, which meant that the acute phase response was not monitored with an acknowledged biomarker. Although serum levels of ferritin were available, the accuracy of ferritin as biomarker has not been established (Beard et al., 2006). The plasma zinc and serum vitamin A levels could therefore be inaccurate in those children suffering from an acute phase response. Although low plasma zinc concentrations may simply reflect an inflammatory state with accompanying acute phase response and not necessarily a suboptimal zinc status, no other accurate markers of zinc status are available (Keen et al., 2004, p. 132). Most other clinical

studies also included plasma zinc and serum vitamin A as markers of micronutrient status. Results from another South African study (Eley et al. 2002) in HIV-infected children, indicated no correlation between CRP levels and the micronutrients measured, which included plasma zinc and serum vitamin A. In this study, it was also evident that multiple micronutrient deficiencies existed – also in relation to those micronutrients not influenced by the acute phase response. The researchers therefore decided to interpret results in relation to vitamin A and zinc in this study with caution.

- Serum folate levels were measured instead of red-cell folate levels which, according to the reference laboratory, means that recent dietary intake of folate could have influenced the outcome. This was confirmed by the fact that none of the children had abnormal low serum folate levels.

7.2.2 Problems in relation to study design

- The researchers had to keep the intervention period to 120 days to exclude the impact of long school holidays. More than half of the children went to family during the two long holidays and it was unclear whether the children would receive regular meals as well as the supplements during that period. It was therefore not possible to determine height growth velocity over a longer, six month period.

- It was not possible to start the intervention period of all the children after precisely the same period after collection of the baseline data. As children from different institutions were screened and included in the study at different times, the intervention took place over two 16 week periods which did not overlap. Unlike the first group, where a four week period occurred between gathering of the baseline data and start of the intervention, ten weeks went by before the second group started the intervention. This occurred as a result from:

- repeated problems to keep the children nil per mouth for collection of the blood samples in one of the centers;
- a sudden breakdown of transport in regard to two of the centers; a small group of children could not be collected from the areas and delivered to the center for an eight week period.

As the budget to redo the blood tests was not available, the researcher and biostatistician had to match the growth and health parameters from before and after the period to ascertain that these few childrens' parameters did not change significantly. The only child with significant changes in the weight-for-age z-score and health status parameters did not complete the intervention period and was lost to follow-up. The researchers therefore believed that the validity of data were not compromised by the event.

- The supplement was only served four times per week, due to the fact that the centres linked to Sunflower House, functioned as day care centres with the children absent during weekends. The recommended 100g dry serving size was however increased to a 150g dry (450g wet) portion, for all the children. This increased portion size was easily consumed by the majority of children. According to the study objectives, it was expected that children would achieve weight gain and benefit in terms of immune and health status. However, it became evident that supplementation of the children's diet on a daily basis with one serving of maize/soy blend, was not sufficient to improve nutritional and micronutrient status in these vulnerable children. Similar results in relation to increased energy intake, associated with enzyme modification in supplemental foods provided to HIV-negative children (Den Besten et al., 1998, Gopaldas & Chinnamma, 1992), could not be achieved in this study. Results from other studies (Den Besten et al. 1998) also indicated that best results could be achieved by providing the modified

supplements as breakfast replacement. Although children were able to consume large portions of the supplement, better results may be achieved by serving smaller portions as energy dense snacks through-out the day, in addition to the normal diet.

- Aggressive nutritional supplementation by means of gastrostomy tube feedings in a sample of 23 HIV-infected children by Miller et al. (1995), showed significant improvements in the weight-for-age Z-scores. In order to facilitate these improvements in weight, the median energy intake was increased from 110kcal/kg/day (462 kJ/kg/day) before enteral supplementation to 189kcal/kg/day (794 kJ/kg/day) during gastrostomy tube feedings. Gastrostomy tube feedings are not available to or affordable for nutrition management of HIV-infected children in developing countries. Supplementation of the diet with maize/soy blends in this study, only increased energy intake from an approximate 350 kJ/kg/day (analysis of institution menu and portion sizes by means of Foodfinder) to an approximate 500 kJ/kg/day. Although the latter is far more than the energy available to the majority of food-insecure, HIV-infected children in the community, it is still well short of the amounts with which successful rehabilitation was done in developed countries. This study once again has shown that HIV-infected children's energy needs far exceed those of HIV-negative children. All the centres which participated in the study had standardised menus, with ration scales, as determined by dietitians of the Department of Health. Even the improved macronutrient intake* of the HIV-infected children during this study, was inadequate to achieve any catch-up growth.

** Replacing breakfast with the maize/soy blends, resulted in increasing the energy intake at breakfast from 1200 kJ to 2600 kJ, a daily RDA in relation to micronutrients in excess of 100% from the supplements alone and a protein intake at approximately 1 g/kg/day from the supplements alone.*

7.3 Conclusions

The following conclusions evolved from the study:

7.3.1 General

- One hundred and fifty five food-secure HIV-affected children in care centres in Mangaung, South Africa, were screened to determine HIV status. Children known to be HIV-infected, as well as those with a positive result of ELISA for anti-HIV antibodies, were purposely sampled and recruited into the study between August 2004 and March 2005. Only 37 tested positive indicating that the majority (75%) of children in care centres in Mangaung is HIV-affected and not infected.

7.3.2 Baseline data

A cross-sectional descriptive study was undertaken on 37 clinically stable, antiretroviral naïve HIV-infected children in these centres.

- Anthropometric results from this study are in accordance with nutritional status data from HIV-infected children in other parts of South Africa (Eley et al. 2002) with 45.9% underweight and 76.7% stunted. HIV-infected children in care centers in Mangaung, do not have a better nutritional status than HIV-infected children screened at health facilities.

- Baseline data regarding the immune status and disease progression were determined from viral loads and CD4 concentrations. The median (quartiles) viral load (n=35) was 117 000 copies/ml (14500; 305 000) and the median (quartiles) $CD4^+$ cell count (n=35) 477 cells/mm^3(300; 791). The median (quartiles) $CD4^+$ percentage was 22.5% (14.2; 35.8). According to the CDC classification, 40%

(14/35) of the group could be categorised in Category 1, with no evidence of immunosuppression. Within the group, 34.3% (12/35) were moderately immunosuppressed (Category 2) and a 25.7% (9/35) severely immunosuppressed (Category 3). From these results, it is evident that at least 25% of the HIV-infected children in care centres in Mangaung, should have access to antiretroviral drugs to optimise management.

- Results from this study have shown that micronutrient deficiencies occurred commonly in the majority of children in care centres, with the highest prevalence of deficiencies relating to glutathione, vitamin A, zinc and vitamin D, which are all essential in maintaining the immune function. Access to regular meals in these centres, did not protect them from developing deficient serum levels of micronutrients.

- Clinical features observed were in accordance with other studies in developing countries, with lymphadenopathy (83.8%), skin rashes (51.4%) and pallor (40.5%) being the most common. Only 8.1% of the children presented with TB, while nutrition related disorders affected only a small number of children. Only six children (16.2%) presented with diarrhoea, while vomiting and oral thrush occurred in less than 10% of the sample. A large group (60%) was anaemic, with iron depletion prevalent in approximately a quarter of the children. Inadequate intake, rather than other nutrition related disorders, may therefore be of greater concern as a possible cause of growth failure.

7.3.3 Correlations between indicators

- Although correlations between immune status and growth only produced weak to moderate results, it is evident that stunted and underweight HIV-infected children in care centers are more likely to have higher viral loads, anaemia and

decreased serum levels of albumin and cholesterol. All of these may impact negatively on disease progression and outcome (Shearer et al. 2000). Despite the high prevalence of micronutrient deficiencies, as well as underweight and stunting, the only indicator which correlated with growth, was glutathione levels.

- Children with abnormal low serum vitamin A and plasma zinc levels, had significantly higher viral loads and lower $CD4^+$ cell counts. As these two indicators could have been influenced by the acute phase response, it is uncertain whether true deficiencies exist. However, results from a study by Eley et al. (2002), showed no correlations between CRP concentrations and micronutrients. Children in this study may therefore be deficient as indicated by the micronutrient concentrations measured. Children with low serum levels of vitamin D, E and glutathione, also presented with higher viral loads and lower $CD4^+$ counts, although the differences were not statistically significant. The presence of micronutrient deficiencies are therefore linked to a compromised immune system, as with data from developed countries.

7.3.4 Impact of nutrition supplementation with an enzyme-modified maize/soy blend

- Children in this study had access to regular, balanced meals including the intervention consisting of a breakfast replacement - an energy dense maize/soy blend, providing in excess of 100% RDA for micronutrients. Despite this intervention, children did not consume more than 400 – 500 kJ/kg/day, which do not reach recommendations by Miller et al. (1995).

- Supplementation of the diet with maize/soy blends in the amounts provided in this study, is clearly not sufficient to establish catch-up growth in antiretroviral naïve, HIV-infected children with growth failure.

- Enzyme-modification in this study did not provide added benefits in terms of growth in children. If energy intake and thus portion sizes were to be adapted to levels to achieve growth, enzyme modification could have played a more important role in lowering the viscosity of the maize/soy blends and preventing early satiety.

- Current initiatives in South Africa, providing supplementation with only soy/maize blends of equal or less quality, are not going to achieve significant results in terms of growth in HIV-infected children.

- A general improvement in the immune status of children supplemented with both maize/soy blends, could be observed from changes in viral loads and $CD4^+$ cell counts. Children in the group consuming the enzyme-modified product demonstrated improvements in $CD4^+$ cell counts approaching significance.

- Viral load reductions equal to those expected of children on ARVs, could not be demonstrated. Only one child in this sample had a viral load reduction of more than 1 log, indicating clinical significance, and only two children improved to become virus undetectable. Nutrition intervention strategies currently available to HIV-infected children in the largest part of South Africa, are inadequate to achieve significant improvements in immune status.

- Despite supplementation of micronutrients in excess of RDA levels, no significant improvements in serum levels of vitamin A, D, E and zinc could be demonstrated.

- Multiple micronutrient supplementation at 100 to 150% RDA for the various micronutrients, as provided to children in this study, did not significantly change serum levels of micronutrients.

- Children consuming the enzyme-modified product were able to maintain serum levels of albumin, opposed to a significant decrease in children on the placebo. This significant better outcome may be explained by the presence of protease in the enzyme mixture, aiding digestion and absorption of protein in the product. As serum albumin was shown to be an important indicator of disease outcome (Shearer et al., 2000; Henderson, 1992), this may be an important outcome in favour of enzyme-modification.

7.4 Recommendations

- The high prevalence of underweight and stunting found at baseline, indicate that inclusion of HIV-infected AIDS orphans in a care centres, do not necessarily protect them from becoming malnourished. It is important to include regular screening of growth indicators into assessment of the children's well-being and not accept that the fact that they receive regular meals, as a guarantee against developing malnutrition.

- Known HIV-infected children in care centres, should receive aggressive nutrition support to support their increased requirements.

- The majority of HIV-infected children presented with micronutrient deficiencies and anaemia as well, which were linked to stunting. In the absence of regular biochemical screening, determination of height-for-age may be a cheap, quick tool to decide which children are in need of further clinical investigations.

- Future studies should include monitoring of biomarkers for acute phase response to control for the presence of acute infection.

- As catch-up growth could not be achieved in this study, additional sources of energy dense supplements such as RTUF and energy drinks should be added to current supplementation regimens to achieve optimal results.

- As nutrition intervention in this study could not demonstrate significant improvements in immune status, it is unlikely that current nutrition intervention strategies will achieve better results in the majority of HIV-infected children in South Africa. Antiretroviral drugs should be made available to all children, including AIDS orphans in care centres, to improve immune function and health status.

- Nutrition supplementation, however, had a general positive effect on the immune status of the majority of the children and therefore, should form part of optimal management of children living with HIV/AIDS.

- As children receiving the enzyme-modified product had better results in term of maintenance of serum albumin levels, further investigation should be done to determine the availability of protein in unmodified maize/soy blends.

7.5 Value of the study

Food insecurity as an underlying cause of malnutrition in HIV-positive children is complicating the interpretation of data following nutrition intervention studies, as exposure of malnourished children to any food and/or supplements may have a positive outcome. By targeting care centers where children have access to regular meals, the researchers were able to exclude food insecurity and to ensure that the impact of the intervention could be measured more accurately. The small sample size as a result from only a few HIV-infected children in these centers, made interpretation of the results very difficult. It however became evident that the majority of HIV-affected children in institutions may well be HIV-negative. The

nutritional and health status of HIV-negative AIDS orphans should be assessed as well, as this may have long-term implications for these children with respect to their development, school achievement and economic productivity later.

Current nutrition intervention strategies for children living with HIV/AIDS are similar to strategies for HIV-negative children with malnutrition. However, random food aid provision with inadequate daily energy value should not be practiced as nutritional care for those infected with HIV (Adamyan, 2004). It is a well known fact that HIV-infected children's energy needs far exceed those of malnourished children. In this study, no benefit in terms of growth or improvement in micronutrient levels could be demonstrated with supplementation amounts per week, equal to the amounts in current regimens in South Africa. In developed countries, rehabilitation of nutritional status in HIV-infected children could be observed if the nutrition assessment and intervention occurred early in the course of the disease (Rothpletz-Puglia, 1998). According to Marston and De Cock (2004), a fine balance is required between the search for "simple" and "appropriate" interventions and efforts to institute measures that health care providers in developed countries take for granted. However, "cost-effective" should not be a polite term for cheap and "simple" should not mean, not effective. HIV-infected children in developing countries, even in care centres, need early, aggressive nutritional support in form of energy dense supplements to complement the diet on a daily basis. The unfortunate reality is that the cost of appropriate nutrition intervention for HIV-infected children in South Africa, may well exceed the cost of ARVs.

The study did contribute to underline the importance of the availability of ARVs to all HIV-infected children, also those in care centres, to aid in weight gain. ARVs should, however, not be seen as the solution to a problem that is far bigger than drug availability. It is also important to address the macronutrient content of supplementation regimens currently available to HIV-infected children. Consensus

about the importance of all the building blocks of good health in HIV, including nutrition support, is of far more importance than provision of drugs alone. According to Ncayiyana (2005), editor of the South African Medical Journal , "...if, in its enthusiasm to blunt dissident views, the medical profession is seduced to overly extol ARVs, trivialise ARV toxicity and pooh-pooh the role of nutrition, the profession may live to regret it."

7.6 References

Adamyan, M. 2004. Strategic use of nutrition interventions for HIV/AIDS prevention and care. Ejournal of the International AIDS Society, Jul11; 1(1): TuPeD5289, Available from: http://www.ias.se/ejias/show.asp?abstract_id=2174809. [Accessed September 9, 2004].

Beard, J.L., Murray-Kolb, L.E., Rosales, F.J., Solomons, N.W. and Angelilli, M.L. 2006. Interpretation of serum ferritin concentrations as indicators of total-body iron stores in survey populations: the role of biomarkers for the acute phase response. American Journal of Clinical Nutrition, 84: 1498 – 1505.

Cant, A.J., Gibb, D.M., Davies, E.G., Cale, C. and Gennery, A.R. 2003. HIV Infection, in Forfar & Arneil's Textbook of Pediatrics. Ed by McIntosh, N., Helms, P. and Smyth, R. 6th ed. Spain: Churchill Livingstone, pp. 1295 – 1309.

Den Besten, L., Glatthaar, I.I. and Ijsselmuiden, C.B. 1998. Adding α-amylase to weaning food to increase dietary intake in children. A randomised controlled trial. Journal of Tropical Paediatrics, 44: 4 – 6.

Eley, B., Sive, A., Kossew, G., Abelse, L., Cooper, M. and Hussey, G. 2002. Growth and micronutrient disturbances in stable, HIV-infected children in Cape

Town. Annals of Tropical Paediatrics, 22: 19 – 23.

Friis, H. and Michaelsen, K.F. 1998. Micronutrients and HIV infection: A review. European Journal of Clinical Nutrition, 52: 157 – 163.

Gopaldas, T. and John, C. 1992. Evaluation of a controlled 6-month feeding trial on intake by infants and toddlers fed a high energy-low bulk gruel versus a high energy-high bulk gruel in addition to their habitual home diet. Journal of Tropical Paediatrics, 38: 278 - 283.

Keen,C.L., Uriu-Adams, J.Y., Ensunsa, J.L. and Gerswin, M.E. 2004. Trace Elements/Minerals and Immunity, in Handbook of Nutrition and Immunity. Ed. by Gershwin, M.E., Nestel, P. and Keen, C.L., New Jersey: Humana Press, New Jersey, pp. 117 – 140.

Marston, B. and De Kock, K.M. 2004. Multivitamins, nutrition, and antiretroviral therapy for HIV disease in Africa. The New England Journal of Medicine, 351: 78 – 80.

Martin, D. 2000. Appropriate laboratory monitoring of HIV. South African Medical Journal, 90(1): 33 – 35.

Miller, T.L., Awnetwant, E.L., Evans, S., Morris, V.M., Vasquez, I.M. and McIntyre, K. 1995. Gastrostomy tube supplementation for HIV-infected children. Pediatrics, 4: 696 – 702.

Ncayiyana, D.J. 2005. Don't let the dissenters distort the medical profession's perspective on treatment for HIV/AIDS. South African Medical Journal, 95(6): 361.

Ramakrishnan, U., Webb, A.L. and Ologoudou, K. 2004. Infection, Immunity and Vitamins, in Handbook of Nutrition and Immunity. Ed. by Gershwin, M.E., Nestel, P. and Keen, C.L., New Jersey: Humana Press, pp. 93 – 116.

Rothpletz-Puglia, P., Oleske, J., Boland, M., Banyai, J., D'Orlando, D. and Dieudonne, A. 1998. Preliminary results of a retrospective study of nutrition intervention strategies for HIV-infected infants and children, International Conference AIDS, 1998; 12: 843 (abstract no. 42344), Available from http:/gateway.nlm.hih.gov/MeetingAbstracts/ 102231142.html. [Accessed September 21, 2006].

Shearer, W.T., Easly, K.A., Goldfarb, J., Jenson, H.B., Rosenblatt, H.M., Kovacs, A. and McIntosh, K. The P^2C^2 HIV Study Group. 2000. Evaluation of immune survival factors in pediatric HIV-1 infection. Annals of the New York Academy of Sciences, 918: 298 - 312.

Appendix A1

UNIVERSITEIT VAN DIE VRYSTAAT
UNIVERSITY OF THE FREE STATE
YUNIVESITHI YA FREISTATA

DEPARTEMENT MENSLIKE VOEDING/DEPARTMENT OF HUMAN NUTRITION
Fakulteit Gesondheidwetenskappe/Faculty of Health Sciences

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2004/04/07

Sr Kgaile
Sunflower House

Dear Sr Kgaile

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 Sunflower 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 supplement s 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 me (cell: 082 8298418).

Thanking you.

Liana Steenkamp

Appendix A2

UNIVERSITEIT VAN DIE VRYSTAAT
UNIVERSITY OF THE FREE STATE
YUNIVESITHI YA FREISTATA

DEPARTEMENT MENSLIKE VOEDING/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 supplement s 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 me (cell: 082 8298418).

Thanking you.

Liana Steenkamp

Appendix B
Ethics committee reference number: 190/00

Consent form

TOESTEMMING / PERMISSION / TUMELLO

TOESTEMMING

Hiermee verklaar ek _____wetlike voog van _____ wat huidiglik permanente/deeltydse versorging by_____ontvang, dat ek my toestemming verleen tot die deelname van _____ aan die navorsingsprojek, soos aan my verduidelik:

Ek is versoek dat _____ 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.
Sewentig HIV+ kinders sal vir 120 dae daagliks in die week een van twee soorte voedingsupplemente in die vorm van verrykte pap ontvang. Alhoewel elke kind slegs op een soort produk is, sal beide produkte voordele vir die kinders inhou.
Bloedmonsters sal van die kinders aan die begin en aan die einde van die projek 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, hoewel ek aangeraai is dat die kind die projek voltooi aangesien dit vir hom/haar voordelig sal wees.
Alle inligting, insluitend HIV status is vertroulik, maar resultate van 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_____, that I give consent that may participate in the project explained to me:

I have been asked that 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.

- Seventy 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 HIV+ status, but the 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: _____ .

Health of Children: Medical examination

Appendix C

Name: _____

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

d d m m y y

Date of examination:_____ [ ][ ][ ][ ][ ][ ] 4-9

Medication:	Yes (1)	No (2)	[ ]	10
If yes, specify1. _____			[ ]	11-12
2. _____			[ ]	13-14
3._____			[ ]	15-16
4. _____			[ ]	17-18
Temperature _____			[ ][ ].[ ]	19-22
Pulse rate _____			[ ][ ]	23-25
Respiratory tempo_____			[ ]	26-27

1. Are any of the following visible/tangible?

Jaundice	Yes (1)	No (2)	[ ]	28
Pallor pale	Yes (1)	No (2)	[ ]	29
Clubbing	Yes (1)	No (2)	[ ]	30
Cyanosis	Yes (1)	No (2)	[ ]	31
Lymphadenopathy	Yes (1)	No (2)	[ ]	32
Oedema	Yes (1)	No (2)	[ ]	33
Skin rash	Yes (1)	No (2)	[ ]	34
Mucosal lesions	Yes (1)	No (2)	[ ]	35
Ear infection	Yes (1)	No (2)	[ ]	36
Loss of appetite	Yes (1)	No (2)	[ ]	37
Weakness	Yes (1)	No (2)	[ ]	38
Fatigue	Yes (1)	No (2)	[ ]	39

2. Are there any respiratory abnormalities?

Dyspnea	Yes (1)	No (2)	[ ]	40
Tachypnea	Yes (1)	No (2)	[ ]	41
Crepitations	Yes (1)	No (2)	[ ]	42
Wheeze	Yes (1)	No (2)	[ ]	43
TB	Yes (1)	No (2)	[ ]	44

3. Is there any abdominal pathology?

Splenomegaly	Yes (1)	No (2)	[ ]	45
Hepatomegaly	Yes (1)	No (2)	[ ]	46
Diarrhoea	Yes (1)	No (2)	[ ]	47
Vomiting	Yes (1)	No (2)	[ ]	48
Oral thrush	Yes (1)	No (2)	[ ]	49

4. Is there any neurological pathology?

Myopathy	Yes (1)	No (2)	☐	50
Neck stiffness	Yes (1)	No (2)	☐	51
Peripheral neuropathy	Yes (1)	No (2)	☐	52
Encephalopathy	Yes (1)	No (2)	☐	53

Comments:_____

Appendix D

Training Manual for Care Centre Staff

August 2004

Index

1. The Basic Food Groups

Food can be divided into 3 basic food groups according to the role the food play in the body.

The food groups are:

- the body-building group;
- the energy group and
- the protective group.

1.1. Body-building Group

The body-building group include: meat, fish, poultry, nuts, eggs and dairy products (such as milk and cheese).

The food in the body-building group contain a substance called **protein**. Protein is the building block of the body, and helps the body to grow and to heal. Body-building foods are very important for children, because the body cannot grow without enough dietary protein. A portion body-building food, equals:

- 1 glass milk;
- 1 egg
- 30g cheese, fish, poultry or chicken (30g = matchbox size portion).

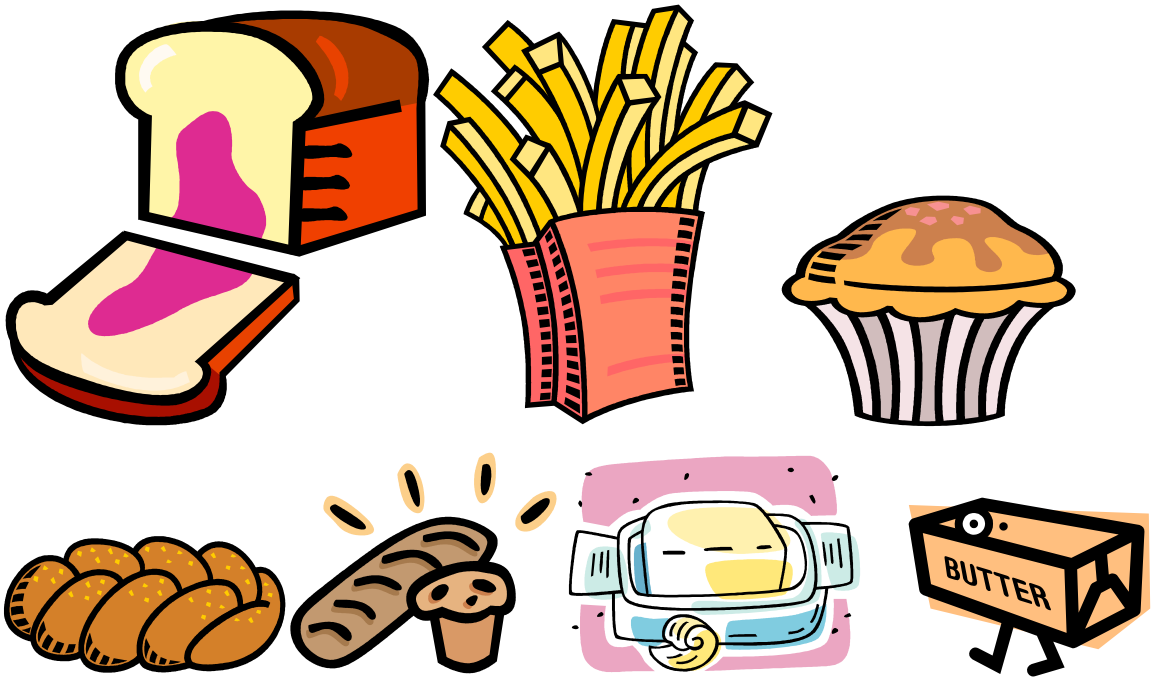
1.2. Energy Group

The energy group supplies the body with energy. Food in the energy 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: oil, butter, margarine, cream, salad dressing, and mayonnaise.

- A portion **fat**, include: 1 teaspoon margarine /butter /oil / mayonnaise
- A portion **starchy** foods are: 1 slice bread
  ½ cup pasta, soft cooked pap
  1/3 cup rice
  ¼ cup mashed potato



1.3. Protective Group

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

A portion **vegetables:** ½ cup cooked vegetables (1/2 cup cooked pumpkin)
1 cup raw vegetables (1 cup carrot salad)

A Portion **fruit**: 1 medium fruit (about the size of a tennis ball)

2. The Importance of Hygiene

Foods contaminated with harmful germs, can cause a series of harmful diseases – called **food borne diseases**. Food borne diseases include food poisoning, food infection, diarrhea and vomiting. The body can loose a lot of fluids through diarrhea and vomiting – causing dehydration. Dehydration can be very serious, and can be **fatal** in young children.

Children with **HIV infection**, have a higher risk to develop food borne diseases. Children with HIV/ AIDS have a depressed immune system, and will not recover as quickly as HIV negative children.

In order to avoid the spread of food borne disease (especially for HIV + children), the following **basic hygiene principals** should be followed:

- always wear a clean uniform/clothes while you are working with food;
- always keep your hair covered with a hairnet or hat – while you are working with food;

- always wash your hands with soap and water before working with food;
- always wash your hands with soap and water after visiting the toilet;

- cover cuts/bruises on your hands with a waterproof plaster;
- if available, wear plastic gloves when you are handling food.
- always wash fruit/vegetables thoroughly before use;
- meat/fish/chicken should be thoroughly cooked; never undercook meat.
- always use fresh food in the kitchen – a golden rule: *if in doubt, throw it out!* one should remember:
- Because children with HIV infection are at a higher risk to develop food borne diseases, left-over food should be avoided if not stored in the fridge!!

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) are different to the porridge in the red packets (referred to as the RED PAP).The porridges should never be mixed and should always be handled apart from each other.

The porridge in the blue packets are referred to as the BLUE PAP, should be mixed in the BLUE MIXING BOWL – and served in BLUE porridge bowls to the children with the BLUE hospital armband.

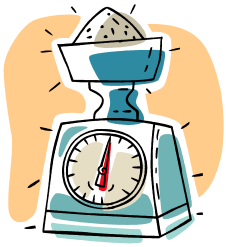
The porridge in the red packets (referred to as RED PAP). should be mixed in the RED MIXING BOWL – and served in RED porridge bowls to the children with the RED hospital armband.

THE MIXING INSTRUCTIONS FOR THE PAP IS DESCRIBED IN 5 STEPS:

Step 1: weighing the pap

- Carefully weigh out the porridge - Every child should receive 150 g DRY PORRIDGE.

- Weigh the total amount of BLUE and RED PAP – the pap should be weighed apart.
- After the amount of BLUE PAP is weighed; the weighed BLUE PAP should be transferred to the BLUE mixing bowl.
- When the RED PAP is weighed, the weighed RED PAP should be transferred to RED MIXING BOWL



Step 2: mixing the pap

- After the pap is weighed, the pap can be mixed.
- For every 150g dry porridge (weighed out per child), you should add 300 ml of very hot water.
- Use boiled water, which has cooled of a bit. It is very important that VERY HOT WATER should be used.

- After the right amount of VERY HOT WATER was added to the porridge, the porridge should be mixed vigorously.

- Let the porridge stand for 5 minutes.

Step 3: Dishing up the pap

- The RED PAP should be dished up in the RED porridge bowls

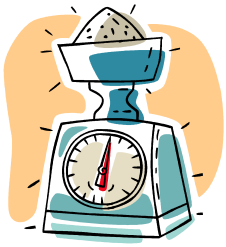
- The Blue PAP should be dished up in the Blue porridge bowls.
- Every child should receive a TOTAL AMOUNT of 412 g RED or BLUE Pap

Step 4: Serving

- Serve every child with the correct amount of porridge – as mentioned in Step 3.
- It is very important that every child receives the correct porridge bowl.
- The porridge bowls are marked in order to help with identification.
- Ensure that every child receive the correct porridge bowl – according to the assigned number.
- Important: a child is only allowed to eat from his/her own porridge bowl. It is not allowed for children to share the porridge – it is not hygienic, children could cross-contaminate each other with harmful germs.

Step 5: Finishing touches!

After the children are finished eating, clear the table. Take the bowls with left-over porridge to the researcher. The researcher will weigh the left over amount of porridge.



4. Completion of Spreadsheet

Value of the spreadsheet:

The spreadsheet will supply the researcher with valuable information necessary for the completion of the study.

Role of Lebone Staff:

The spreadsheet is a form that should be completed after every meal for all the children included in the study.

The fieldworker should evaluate the children's plates after every meal, and fill in a spreadsheet to help the researcher.

The spreadsheet will only consist of a few easy questions, with only **yes / no** answers.

Spreadsheet:

Date: …28 August…… Group: …… Group 1 : Blue Porridge…….

	Any Left-overs? Yes/ no				
Name:	**Porridge**	**Snack**	**Lunch**	**Snack**	**Supper**
Nancy	**Yes**	**No**	**No**	**No**	**No**
Maria	**No**	**No**	**No**	**Yes**	**Yes**

5. Importance of not taking any other supplements

During the length of the study, it is important that children should not receive ANY other forms of supplements.

"Other" types of supplements include:
multivitamins, multivitamin- syrups, e-pap, etc.

Why no other supplements?
The Blue Pap and the Red Pap contains big amounts of added vitamins and minerals. If children receive too much vitamins and minerals, it could be potentially dangerous. It is in the interest of the children that we are prohibiting the use of other supplements during the length of this study.

6. Health Indicators Questionnaire

Dr. Walsh is a medical practitioner who will come and examine the children on a monthly basis. It is important, however, that the children's health should be monitored on a daily basis – in order to supply the doctor with more information during the examinations.

It is requested that a questionnaire should be completed for every child on a daily basis. The questionnaire is very easy, and will only take a few minutes to complete.

If a child is ill, you must mark/tick the specific symptom on the spreadsheet – on the specific day of the week.
If a child is given medication, just fill in the name of the medication on the questionnaire.
If a child has fever, specify the temperature – and fill in on the spreadsheet.

SUMMARY

The aim of the study was to describe changes in growth, immune status, micronutrients linked to immune status and health status in food secure HIV-infected children, after nutrition intervention with an enzyme modified maize supplement. Sub-objectives included the determination of baseline data with regard to growth, immune and health status and to determine correlations between these indicators.

One hundred and fifty five food-secure HIV-affected children in care centres in Mangaung were screened to determine HIV status. A randomised, double blind, clinically controlled, prospective trial was subsequently undertaken on 37 clinically stable, antiretroviral naïve, HIV-infected children. All the children received co-trimoxazole prophylaxis and were dewormed prior to the intervention.

Baseline data showed a median age of 5.35 years within the group. Fifteen children (46%) were underweight, 30 children (77%) were stunted and one child was wasted. The median viral load of the group was 117 000 copies/ml, with the median $CD4^+$ cell count and percentage, 477 cells/mm^3 and 22.5% respectively. Multiple micronutrient deficiencies were found amongst the children in relation to glutathione in 91%, albumin in 78%, vitamin A in 63%, vitamin D in 44%, zinc in 38% and vitamin E in 13%. Sixty percent of the children were anaemic and 30% were iron deficient. The most commonly occurring clinical features were lymphadenopathy in 84%, skin rashes in 51% and hepatomegaly in 32%. Nutrition related disorders presented in less than 20% of the sample.

Significant but weak positive correlations were demonstrated between the height-for-age z-score (HAZ) and the CD4%, serum cholesterol and -albumin. Apart from a significant but weak positive correlation between the weight-for-height z-score (WHZ) and

glutathione, no other correlations could be demonstrated between micronutrient levels and growth. Viral load was significantly but weakly to moderately negatively correlated with the CD4:CD8, CD4%, $CD4^+$ cell count, haemoglobin, serum albumin, -cholesterol, -vitamin A, -vitamin D and -zinc. Significantly weak to moderate positive correlations were reported between CD4 concentrations and serum albumin, -hemoglobin, -cholesterol, -vitamin A, -vitamin D, -zinc and -glutathione. High viral loads and decreased CD4 concentrations in food secure children in care centres are linked to decreased serum levels of micronutrients, albumin and haemoglobin.

Nutrition supplementation with two maize soy blends (experimental and placebo) with exactly the same content, apart from the enzyme mixture in the experimental product, resulted in the following: Non-significant decreases in median viral loads of 30 145 copies/ml for the experimental group (E) and 350 copies/ml for the placebo group (P). The median (quartiles) $CD4^+$ cell count increases were 38.5 (-17; 180) cells/mm^3 for E ($p=0.07$) and 167 (-160; 575) cells/mm^3 for P ($p=0.76$). No significant differences relating to growth and changes in micronutrient levels could be demonstrated between the two groups. Children in P experienced a significant ($p=0.003$) decrease in serum albumin levels, while the E group maintained their levels, resulting in a significant difference.

Nutrition supplementation in both groups led to non-significant improvements in median viral loads and $CD4^+$ cell counts. Improvements could, however, not reproduce decreases in viral loads expected from children on ARVs. There was a significant difference between the two groups in relation to changes in serum albumin levels. As serum albumin levels are linked to higher $CD4^+$ cell counts in most studies, this outcome need to be further investigated. None of the groups benefited in terms of growth, health status or micronutrient status, despite micronutrient intakes in excess of 100% RDA. The high

prevalence of underweight and stunting found at baseline indicate that inclusion of HIV-infected AIDS orphans in care centres, do not necessarily protect them from becoming malnourished. Known HIV-infected children in care centers should receive optimal care by including ARVs and more aggressive nutrition support into treatment regimens.

Paediatric; HIV; Nutrition intervention; Micronutrients; Immune; Viral load; Maize/soy blends; Supplementation

OPSOMMING

Die doel van die studie was om veranderinge in groei, immuunstatus, mikronutriënte wat met immuunstatus verband hou en gesondheidstatus in MIV+ kinders te bepaal, na supplementasie met ‘n ensiemgemodifiseerde mieliepapsupplement. Die sub-doelwittte was om basislyndata rakende groei, immuun- en gesondheidstatus in hierdie HIV+ kinders, wat in sentrums versorg word te bepaal, asook om korrelasies tussen die onderskeie indikatore vas te stel.

Honderd-vyf-en-vyftig kinders deur MIV geaffekteer, in sentrums in Mangaung, is getoets om MIV-status te bepaal. Die 37 MIV+ kinders (nog nooit aan antiretrovirale middels blootgestel nie), is vervolgens ingesluit in ‘n gerandomiseerde, dubbelblinde, klinies gekontroleerde, prospektiewe proef oor ‘n periode van 120 dae. Die hele groep het *co-trimoxazole* profilakse ontvang en is voor die intervensie ontwurm.

Basislyndata het getoon dat die mediaan ouderdom van die kinders 5.35 jaar was. Vyftien kinders (46%) was ondermassa vir ouderdom, 30 (77%) se lengtegroei was ingekort en een kind was wangevoed. Die mediaan virale telling was 117 000 kopieë/ml, met die mediaan $CD4^{+}$-seltelling en persentasie 477 selle/mm^3 en 22.5% onderskeidelik. Veelvuldige mikronutriënttekorte was in die meeste kinders teenwoordig en wel m.b.t. glutatioon (91%), albumien (78%), vitamien A (63%), vitamien D (44%), sink (38%) en vitamien E (13%). Sestig persent van die kinders was anemies en 30% het met ‘n ystertekort presenteer. Die mees algemene kliniese tekens was limfadenopatie in 84%, veluitslag in 51% en hepatomegalie in 32% van die kinders. Voedingverwante simptome was in minder as 20% van die kinders aanwesig.

Betekenisvolle, dog swak positiewe korrelasies is tussen lengte-vir-ouderdom en die CD4%, serumcholesterol en –albumien aangetoon. Behalwe vir ‘n betekenisvol swak positiewe korrelasie tussen massa-vir-lengte en glutatioonvlakke, kon geen ander korrelasies tussen groei en mikronutriënte gevind word nie. Die virale tellings het betekenisvolle, maar swak tot matige negatiewe korrelasies met die CD4:CD8, CD4%, $CD4^+$ seltelling, hemoglobien, serumalbumien, -cholesterol, -vitamien A, -vitamien D en -sink getoon. Betekenisvolle swak to matige positiewe korrelasies is gerapporteer tussen CD4 konsentrasies en serumalbumien, -hemoglobien, -cholesterol, -vitamien A, -vitamien D, -sink en -glutatioon. Hoë virale tellings en verlaagde CD4 konsentrasies in kinders in versorgingsentrums kan verbind word aan verlaagde mikronutriëntvlakke, asook verlaagde albumien en hemoglobien.

Voedingsupplementasie met twee mielie/soja mengsels (eksperimentele (E) en plasebo (P) produk) met identies dieselfde inhoud, behalwe vir die ensiemmengsel in die eksperimentele produk, het die volgende resultate tot gevolg gehad: Nie-betekenisvolle afnames in die mediaan virale tellings van 30 145 kopieë/ml vir die groep op E en 350 kopieë/ml vir die groep op P is gedemonstreer. Die mediaan (kwartiele) $CD4^+$-seltellings het met 38.5 (-17; 180) selle/mm^3 vir die groep op E (p=0.07) en 167 (-160; 575) selle/mm^3 vir die groep op P (p=0.76) toegeneem. Geen betekenisvolle verskille kon aangetoon word rakende groei en mikronutrientindikatore tussen basislyn en eindwaardes nie. Kinders op die plaseboproduk het ‘n betekenisvolle (p=0.003) verlaging in serumalbumienvlakke ondervind, terwyl kinders op die eksperimentele produk hul albumienvlakke in stand gehou het.

Voedingsupplementasie in beide groepe het tot nie-betekenisvolle verbeterings in virale tellings en CD4-konsentrasies aanleiding gegee. Dié verbeterings was egter heelwat

swakker as die resultate wat verwag word wanneer kinders op antiretrovirale middels geplaas word. ‘n Betekenisvolle verskil tussen uitkomste rondom serumalbumienvlakke is tussen die twee groepe aangetoon. Aangesien serumalbumien met immuunstatus verbind word, behoort hierdie resultate verder ondersoek te word. Geeneen van die groepe het voordeel getrek in terme van groei, verbetering van mikronutriëntstatus of gesondheidstatus nie, ten spyte van ‘n mikronutriëntinname wat 100% van die Aanbevole Daaglikse Toelating oorskry het. Die hoë persentasie kinders met ondervoeding dui daarop dat insluiting van VIGS-wesies in versorgingsentrums nie noodwendig kinders teen die ontwikkeling van wanvoeding beskerm nie. Bekende MIV+ kinders in versorgingsentrums behoort optimale behandeling te ontvang deur die beskikbaarstelling van antiretrovirale middels asook aggressiewe voedingondersteuning.

Pediatrie; MIV; Voedingintervensie; Mikronutriënte; Immuunstatus; Virale telling; Supplementasie; Mielie/Soja mengsels