Multi-micronutrient supplementation in HIV-infected South African children

Effect on nutritional status,
diarrhoea and respiratory infections

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Abstract

Background: The nutritional status of HIV-infected children is reported to be poor. Diarrhoea and acute respiratory infections tend to be more common and severe in HIV-infected children than in uninfected ones. Deficiencies of micronutrients may result in poor growth and increased risk of diarrhoea and respiratory infections. Micronutrient deficiencies are common in HIV-infected children. The poor growth, diarrhoea and respiratory infections seen in HIV-infected children may be partly due to micronutrient deficiencies. The studies in this thesis had two main objectives: (1) to evaluate the effect of short-term (during hospitalization) and long-term (6 months) multi-micronutrient supplementation on episodes of diarrhoea and respiratory infections in HIV-infected children who are not yet on antiretroviral therapy (ART), and (2) to assess the effects of long-term multi-micronutrient supplementation on appetite and growth performance of HIV-infected who are not on ART.

Methods and results: Four studies were conducted. Initially a cross-sectional study was performed in which the duration of hospitalization, weight, length, micronutrient status and appetite of HIV-infected children admitted with diarrhoea or pneumonia was compared with the results of HIV-uninfected children. Duration of hospitalization was 2.8 days (52%) longer in HIV-infected children. Appetite as measured by amount of test food eaten (g per kg body weight) was 26% poorer in HIV-infected children. Mean length-for-age Z-scores were lower in HIV-infected children; there was no difference in level of wasting.

Subsequently multi-micronutrient supplementation studies were performed, one short-term and two long-term studies. The effect of supplementation on the duration of hospitalization in HIV-infected children with diarrhoea or pneumonia was assessed in the short-term study. One long-term study assessed the supplement's impact on growth and frequency of episodes of diarrhoea and of pneumonia in HIV-infected children. The other evaluated the effect of the supplement on the appetite of these children. The supplement contained vitamins A, B complex, C, D, E and folic acid, and the minerals copper, iron, selenium and zinc at levels based on recommended dietary allowances.

In the short-term supplementation study HIV-infected children aged 4-24 months who were hospitalized with pneumonia or diarrhoea received the supplement or a placebo until discharge from hospital. The duration of hospitalization was 1.7 days (19%) shorter in the supplement group.

Long-term multi-micronutrient supplementation improved the weight-for-age and weight-for-height Z-scores of HIV-infected children aged 4-24 months by 0.4 over the 6-month period. There was no improvement in stunting. Children in the supplement group had substantially fewer episodes of respiratory symptoms per month than the placebo group (0.66 ± 0.51) per month vs (1.01 ± 0.67) ($P < 0.05$) and marginally fewer episodes of diarrhoea per month (0.25 ± 0.31) vs (0.36 ± 0.36) ($P = 0.09$). There was no effect on CD4 lymphocytes. Long-term supplementation with micronutrients had benefits on the appetite of HIV-infected children aged 6-24 months as well. Improvements in amount of test food eaten over the 6-month period were much higher among children who received the supplement (4.7 ± 14.7 g/kg body weight) than the changes in those who received the placebo (-1.4 ± 11.6 g/kg body weight).

Conclusion: Multi-micronutrient supplementation reduces the duration of diarrhoea and of pneumonia and incidence of diarrhoea and of respiratory symptoms in HIV-infected children who are not yet on ART. Multi -micronutrient supplementation also improves appetite and weight in these children but not height. The results of these studies indicate that multi-micronutrient supplementation should be considered in HIV-infected infant and young children who have not commenced ART.

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Introduction

Chapter 1

INTRODUCTION

In spite of numerous advances that have been made in relation to the treatment and indeed the prevention of HIV infection in children, it remains a major problem among children in sub-Saharan Africa. It is estimated that there were 2.5 million children worldwide living with HIV at the end of 2009, 2.3 million of them in sub-Saharan Africa (UNAIDS, 2010). The mortality and morbidity associated with HIV infection in children is quite high; the number of child deaths due to HIV in 2009 is estimated to be 280 000 (UNAIDS, 2009). HIV infection is thought to be a cause in 42% of under-5 child deaths in South Africa, in 2004 (Statistics South Africa, 2006). Common clinical manifestations in HIV-infected children include severe malnutrition and diarrhoea and respiratory infections (Pol *et al.* 2007). Micronutrient deficiencies are common among children in developing countries (Micronutrient Initiative, 2004), but these deficiencies are even more common and more severe among HIV-infected children (Semba & Tang 1999). Micronutrient deficiencies on their own are associated with poor growth and increased susceptibility to diarrhoea and respiratory infections (Semba & Tang 1999). The research that is described in this thesis focuses on two major issues. The first is the impact of short and long-term multi-micronutrient supplementation on episodes of diarrhoea and respiratory infections in HIV-infected children. The second issue is the effect of long-term multi-micronutrient supplementation on appetite and growth performance of HIV-infected children.

In this chapter, a summary of what is known on the nutritional status of HIV-infected children is discussed. Subsequently, the rationale for multi-micronutrient supplementation in HIV-infected children will be elucidated, followed by a discussion on the public health relevance of such supplementation in the South African setting. At the end of the chapter the objectives of the thesis research will be described and the outline of the thesis explained.

HIV-infected children and their nutritional status

Failure to thrive has been a prominent feature of paediatric HIV since the early cases were observed (Arpadi, 2000), and it's thought to be an indicator of HIV disease progression (Arpadi, 2005). There is also a strong association between micronutrient deficiency, poor

growth and HIV disease progression (Semba & Tang, 1999). In this section therefore we shall briefly discuss the magnitude of HIV disease in children, followed by the growth performance (weight and height) of HIV-infected children and the micronutrient status of these children.

Magnitude of HIV disease in children

The number of children under 15 years living with HIV worldwide in 2009 was estimated to be 2.5 million, and 90% of these children were in sub-Saharan Africa (UNAIDS, 2010). The number of children younger than 15 years infected with HIV in South Africa was estimated to be 280 000 (UNAIDS, 2010). In terms of new infections with HIV it is estimated that there were 430 000 children younger than 15 years worldwide who were newly infected with HIV in 2008, and 90% of them were in Sub-Saharan Africa (WHO, 2010). In South Africa the number of children under 14 years of age who were newly infected with HIV in 2006 was approximately 64 000 (Dorrington *et al.* 2006). Over 90% of HIV infections in children are due to mother-to-child transmission (WHO, 2010).

Infection with HIV has a major impact on the admission of children to South African hospitals (Rabie *et al*. 2007), and the infection has changed the profile of paediatric admission diagnoses and has increased the mortality rate of hospitalized children (van Deventer *et al*. 2005). A South African academic hospital reported that approximately 60% of children admitted were HIV-infected (Pillay *et al*. 2001). A survey that reviewed the mortality in 16 South African hospitals showed that approximately 48% of the deaths of children admitted to these hospitals were related to HIV infection (Patrick & Stephen, 2008).

HIV infection is thus a major problem among South African children; therefore interventions that can mitigate its effects are crucial.

Growth performance of HIV-infected children

Poor nutritional status, pneumonia and diarrhoea have been noted as the most common causes of death in HIV-infected children (Patrick & Stephen, 2008). Slow weight gain and poor linear growth are common manifestations of paediatric HIV infection (Chantry *et al*. 2003). A strong inverse relationship between the rate of growth and the degree of HIV viral replication has been reported (Arpadi *et al*. 2000).

The postnatal growth of HIV-infected children, as measured by weight-for-age and length-for-age has been observed to be poorer than that of HIV-exposed but uninfected children; this has been observed in both developed and developing countries (Isanaka *et al*. 2009). A study that prospectively followed children born to HIV-infected mothers in 11 European countries revealed that infected children had poorer growth (in terms of both weight and height) compared to uninfected children (Newell *et al*. 2003). The prevalence of moderate to severe underweight and stunting among HIV-infected Indian children was noted to be 63% and 58%, respectively (Padmapriyadarsini *et al.* 2009). Similarly, the prevalence of underweight and stunting was shown to be significantly higher in HIV-infected Ugandan children than in uninfected ones (Nalwoga *et al.* 2010). Impairment in weight-for-age and length-for-age has been detected as early as 3-4 months after birth (Isanaka *et al*. 2009). The weight and height impairment seems to worsen with increasing age (Arpadi, 2005; Newell *et al*. 2003).

Micronutrient status of HIV-infected children

Micronutrient deficiencies have been shown to be more severe in HIV-infected children, compared to uninfected children, and several micronutrient deficiencies often coexist (Eley *et al.* 2002a; Eley *et al.* 2002b). In a study that measured the concentrations of vitamins A, B6, B12, E and folic acid, zinc and copper in stable antiretroviral naïve HIV-infected South African children whose median age was 25 months, 62% had two or more deficiencies of these micronutrients (Eley *et al.* 2002a). Zinc deficiency was also found to be frequent among HIV-infected Ugandan children; where it was observed that two thirds of untreated (with antiretroviral therapy (ART)) children were zinc deficient (Ndeezi *et al.* 2010). Anaemia and iron deficiencies have also been noted to occur frequently in stable HIV-infected children; in a group of HIV-infected South African children, 73% were anaemic and 45% of all anaemic children were iron depleted (Eley *et al.* 2002b). A review by Singhal and Austin (2002) indicated that deficiencies of vitamins B2, B6, B12, E and folic acid are common in HIV-infected persons.

Role of micronutrients in the immune system

Zinc deficiency affects both non-specific and specific immunity. Deficiency of zinc results in epidermal cell damage, and damage to the linings of the gastrointestinal and pulmonary tracts

has been observed during zinc deficiency (Finamore *et al*. 2008; Prasad, 2007). Deficiency of zinc also affects other mediators of non-specific immunity, such as polymorphonuclear leucocyte and natural killer cell function (Finamore *et al*. 2008; Prasad, 2007). The cytokines interleukin-1, interleukin-2 (IL-1, IL-2) and interferon-γ have been reported to be suppressed during zinc deficiency (Prasad, 2008). Zinc deficiency results in decreased T and B lymphocyte concentrations and depressed T and B lymphocyte functions, and decreased CD4+:CD8+ ratios are also seen (Prasad, 2007; Prasad, 2008). Zinc is an antioxidant and its role in modulating oxidative stress has been recently recognised (Prasad, 2008). Children with low plasma zinc levels have been observed to have an increased risk of episodes of diarrhoea and acute respiratory infections compared to children with normal plasma zinc levels, and zinc deficiency is thought to result in a substantial disease burden among children less than 5 years of age (Fischer Walker *et al*. 2009).

Vitamin A plays an important role in maintaining the structural and functional integrity of mucosal epithelial cells and this is essential in preventing microbial invasion (Mehta & Fawzi, 2007). Loss of integrity of the epithelial lining of mucous membranes in vitamin A deficient persons explains the close association with increased susceptibility to gastrointestinal and respiratory infections (Mehta & Fawzi, 2007). Indeed it has been indicated that children with mild vitamin A deficiency are at increased risk of both diarrhoeal and respiratory disease (Nojilana *et al*. 2007). The functions of vitamin A include increasing the levels of acute phase reactants in response to infection, regulating monocyte function, improving the cytotoxicity of natural killer cells and increasing total lymphocyte, especially the CD4 counts (Mehta & Fawzi, 2007).

Impairment of cell mediated immunity has been described in iron-deficient humans (Kumar & Choudhry, 2010). Iron deficiency has been demonstrated to reduce the bactericidal effects of neutrophils (Bhaskaram, 2002). Other immunological abnormalities that have been observed with iron deficiency include impaired natural killer cell activity, reduced production of macrophage inhibitor activity, depression of T-lymphocyte numbers, defective T-lymphocyte induced proliferative response, impaired interleukin-2 production by lymphocytes and impairment of delayed cutaneous hypersensitivity (Kumar & Choudhry, 2010). On the other hand, iron is essential for both the child and the invading pathogen (Ianotti *et al.* 2006),

but iron binding proteins such as transferrin and lactoferrin inhibit bacterial growth by making iron unavailable to the pathogens (Oppenheimer, 2001).

Micronutrients such as vitamin C, vitamin E, selenium, copper and riboflavin are powerful antioxidants and antioxidant deficiencies have been found to adversely influence the cytokine profile of host T cells (Bhaskaram, 2002). Vitamin B-6 deficiency is associated with reduced lymphocyte proliferation and antibody production, while neutrophil function is reduced with vitamin B12 deficiency (Dreyfuss & Fawzi, 2002). Riboflavin deficiency impairs the ability to generate humoral antibodies in response to antigens (Fawzi *et al.* 1999). Vitamin E is known to influence the functions of T and B cells, as well as phagocytes (Semba & Tang 1999). Vitamin C deficiency has been associated with both B and T cell lymphocyte dysfunctions (Fawzi *et al.* 1999). Selenium deficiency inhibits neutrophil function, the cytotoxicity of T lymphocytes and natural killer cells, and it also inhibits antibody production (Dreyfuss & Fawzi, 2002).

There are many potential causes of poor nutritional status (growth performance and micronutrient status) in HIV-infected children, and these include reduced dietary intake, increased nutrient losses and increased nutrient requirements.

Reduced dietary intake

Decreased oral intake in HIV-infected children results from various causes including anorexia (loss of appetite), dysphagia (difficulty in swallowing) and odynophagia (painful swallowing (Semba & Tang, 1999). Loss of appetite has been reported to be common among HIV-infected children (Heikens *et al*. 2008). The loss of appetite is probably a major contributor to the decreased dietary intake. The dysphagia and odynophagia may be caused by oro-pharyngeal candidiasis which is a frequent finding in HIV-infected children (Singh *et al*. 2003).

Increased nutrient losses

Malnutrition can result from increased nutrient losses as a result of chronic vomiting, diarrhoea and malabsorption. Malabsorption has been noted to be more common in HIV-infected children and is associated with advanced HIV disease and higher degrees of malnutrition (Densupsoontorn *et al*. 2009). Diarrhoea and malabsorption of fats and carbohydrates appear to be common in all stages of HIV infection (Semba & Tang, 1999).

Malabsorption of fat probably results in reduced absorption of fat-soluble vitamins such as A, D, E and K.

Increased nutrient requirements

The utilization of nutrients (including micronutrients) is thought to be increased in HIV-infected children (Duggan & Fawzi, 2001). The increased utilization is partly related to the metabolic effects of cytokines released and inflammation associated with HIV infection (Keusch & Farthing, 1990). Catabolism of stored nutrients is necessary for the provision of energy and substrates required for the increased protein synthesis of the normal host response to infection (Keusch & Farthing, 1990).

It has been postulated that total energy expenditure is increased in children with HIV, and this may be secondary to increases in resting energy expenditure (REE). REE (adjusted for fat free mass) has been reported to be increased in HIV-infected adults when compared to uninfected ones (Fitch *et al*. 2009). In a study conducted in American children, the mean REE was lower in HIV-infected children than in uninfected children, however, the mean REE/kg of body weight was significantly higher among HIV-infected children (Henderson *et al*. 1998). A higher energy expenditure implies an increase in requirements of nutrients.

In summary, the nutritional status of HIV-infected children has been noted to be poor in terms of weight, height and micronutrients. The causes of the poor nutritional status include poor food intake (which is partly related to appetite), malabsorption and increased requirements of nutrients.

Relationship between micronutrient levels and HIV infection

Deficiency of micronutrients and HIV infection are both individually associated with immune deficiency and HIV infection itself results in micronutrient deficiency. The deficiency of micronutrients in HIV-infected children is partly related to poor dietary intake (poor appetite, mouth sores etc), malabsorption of nutrients (including micronutrients) and increased requirement of nutrients. The resultant micronutrient deficiency will further suppress immunity and will accelerate the progression of HIV disease. This will increase susceptibility to infections (including diarrhoea) even more, with additional loss of micronutrients. This vicious cycle is well described in the review by Semba and Tang (1999).

It has thus been noted that deficiencies of micronutrients are common in HIV-infected children, and that these children commonly lack multiple micronutrients. Furthermore, these micronutrients play an important role in the immune system thus their deficiency is likely to increase susceptibility to infections.

Rationale for multi-micronutrient supplementation in HIV-infected children

People in the developing world show deficiencies of not just one micronutrient, but of several key micronutrients including vitamins A, B2, B12, and folic acid, iron and zinc (Micronutrient Initiative, 2004). The most prevalent micronutrient deficiencies include vitamins A, B2, B12, D and E, and iron and zinc; deficiencies of these micronutrients often occur together due to a low intake of animal source foods (Allen *et al.* 2009; Murphy & Allen, 2003). HIV-infected children have been shown to have multiple micronutrient deficiencies (Eley *et al.* 2002a; Eley *et al.* 2002b). In view of the fact that micronutrient deficiencies commonly occur as concurrent deficiencies of several micronutrients, it is considered that the deficiencies should be addressed simultaneously (Gross *et al.* 2005).

A number of studies have assessed the effects of multi-micronutrient supplementation on growth and duration and number of episodes of diarrhoea and of pneumonia in children. Most of these studies were performed in children who were not HIV-infected, however, a large number of the children were undernourished, and as previously stated HIV-infected children tend to be undernourished. These studies may thus be relevant for HIV-infected children. While there is a number of studies that have looked at the effects of multi-micronutrient supplementation, a larger number of studies assessed the effects of a single micronutrient. When deciding which micronutrients should be included in a multi-micronutrient supplement, it is useful to assess the effects of individual micronutrients on these children as well.

Effects of micronutrient supplements on appetite

Deficiency of zinc has been associated with taste disturbances and poor appetite (Ueda *et al*. 2006; Chen *et al*. 2000). It is also thought that zinc levels may have an effect on serum leptin (a hormone involved in the regulation of appetite) concentrations (Kwun *et al*. 2007).

Supplementation with zinc was shown to improve the appetite of zinc deficient patients with cystic fibrosis (Van Biervliet *et al.* 2008).

Iron deficiency is also thought to be associated with poor appetite (Isguven *et al.* 2007), and iron supplementation might improve appetite. Indeed the appetites of Zanzibari school children were improved by supplementation with iron (Stoltzfus *et al.* 2004).

Supplementation with a multi-micronutrient (containing vitamins A, B1, B2, B3, B5, B6, B12, C, D, E and folic acid and zinc, iron, copper, iodine, manganese, chromium, selenium and molybdenum) for six weeks did not improve the appetite of children in Benin (a country with low HIV prevalence) (Dossa *et al*. 2002). Adding more iron to the afore-mentioned supplement did not improve the appetite of Beninese children (Dossa *et al*. 2001).

The benefits of multi-micronutrient supplementation on appetite have not been clearly established, and as far as we know there is no published study that assesses the effects of multi-micronutrient supplementation on appetite of HIV-infected children. We therefore decided to design a study that assesses the effect of multi-micronutrient supplementation on the appetite of HIV-infected children.

Effects of micronutrient supplements on growth

Zinc, vitamin A and iron deficiencies are known to be associated with impaired growth (Brown *et al*. 2002; Umeta *et al*. 2003; Melikian *et al*. 2001; Isguven *et al*. 2007). Zinc supplementation may significantly improve growth in prepubertal children (Brown *et al*. 2002). Supplementation with iron for six months to a group of malnourished Indian children resulted in significantly higher gains in weight and in height compared to children who were given a placebo (Abdelrazik *et al*. 2007). Multi-micronutrient supplementation (vitamins A, B1, B2, B3, B6, B12, C, D, E and folic acid, and zinc, copper, iodine and iron) was shown to be beneficial for growth in Vietnamese children (Hop *et al*. 2005). Likewise supplementation with a multiple micronutrient (containing vitamins A, B1, B2, B3, B6, B12, C, D, E and K, and folic acid, zinc, copper, iodine and iron) improved length-for-age in stunted HIV-uninfected South African children, compared to children given vitamin A alone or zinc with vitamin A (Chhagan *et al*. 2010). However, multiple micronutrient supplementation had no effect in on the linear growth of another group of South African children (HIV status not indicated) (Smuts *et al*. 2005).

Effects of micronutrient supplements on diarrhoea

Diarrhoea has been reported to be significantly more frequent and more severe among HIV-infected than among uninfected children (Humphreys *et al*. 2010). Certainly, diarrhoeal disease has been noted as the second most common admission diagnosis among HIV-infected South African children (van Deventer *et al*. 2005).

The aetiology of HIV-related diarrhoea is multi-factorial and is thought to include opportunistic gut infections (due to suppressed immunity) and intestinal dysfunction (Janoff & Smith, 2001; Keusch & Farthing, 1990). HIV infection itself has a major impact on the structure and function of the alimentary tract, and the virus has been found in the intestinal mucosa and may directly lead to intestinal symptoms (Keusch & Farthing, 1990). The transactivating peptide (Tat), which is produced by the HI virus, is thought to play a primary role in pathogenesis of diarrhoea in HIV-infected patients (Canani *et al*. 2007). Tat stimulates active fluid secretion into the gut lumen and inhibits fluid absorption at the intestinal level (Canani *et al*. 2003; Canani *et al*. 2006).

Zinc supplementation in children with acute diarrhoea has been shown to reduce the duration of diarrhoea, stool output and the risk of prolonged diarrhoea (Bhatnagar *et al*. 2004). The beneficial effects of zinc treatment on the duration of diarrhoea have been summarised in the review by Hoque and Binder (2006), and are thought to include the following: (a) improved absorption of water and electrolytes by the intestines, (b) faster regeneration of gut epithelium, (c) increased levels of enterocyte brush-border enzymes, and (d) an enhanced immune response leading to an increased clearance of the pathogen/s responsible for diarrhoea from the intestines. Zinc has also been shown to have an inhibitory effect against HIV-related Tat protein induced secretory diarrhoea thus directly limiting a specific mechanism of HIV-related diarrhoea (Canani *et al*. 2006). Supplementation with zinc also seems to reduce the incidence and severity of diarrhoeal episodes in children (Bhandari *et al*. 2002). A reduced incidence of diarrhoea was observed in zinc supplemented HIV-infected South African children (Bobat *et al.* 2005).

Vitamin A supplementation seems to be associated with protective effects in HIV-infected children with diarrhoea. The risk for severe watery diarrhoea has been observed to be lower in HIV-infected children supplemented with vitamin A (Mehta & Fawzi, 2007).

Vitamin A supplementation was associated with a trend towards decrease in persistent diarrhoea among HIV-infected Ugandan children (Semba *et al*. 2005).

Similarly, supplementation with a multi-micronutrient was shown to reduce the incidence of diarrhoea in stunted HIV-uninfected children (Chhagan *et al.* 2009). Another study on the other hand revealed no effect on diarrhoeal frequency or severity in HIV-infected and uninfected South African children who received a multi-micronutrient (Luabeya *et al.* 2007).

Effects of micronutrient supplements on pneumonia

The HIV epidemic has markedly increased the incidence and severity of childhood pneumonia in developing countries and approximately 90% of HIV-infected children will develop a respiratory illness during the course of their HIV disease (Graham, 2003; Graham, 2007). The incidence of pneumonia has been shown to be more common in HIV-infected than in uninfected children (Madhi *et al*. 2000). Respiratory diseases are the major cause of morbidity and mortality in HIV-infected children (Graham, 2003). A study conducted in Johannesburg, South Africa, indicated that although the prevalence of HIV-1 infection is 5% among children younger than 5 years; children in this age group accounted for 45% of those hospitalized and 85% of those who died as a result of severe pneumonia (Madhi *et al*. 2000).

Supplementation with zinc may be beneficial in reducing the duration of pneumonia (Brooks *et al*. 2004), but this is not a consistent finding (Bose *et al*. 2006). A meta-analysis concluded that zinc supplementation reduces the risk of respiratory illness (Aggarwal *et al*. 2007).

While some studies have suggested that vitamin A may reduce the duration of non-measles pneumonia (Julien *et al*. 1999), a meta-analysis indicated that there is no evidence that high-dose Vitamin A (≥ 100 000IU for infants and ≥ 200 000 IU for children older than 1 year) improves recovery from pneumonia in developing countries (Brown & Roberts, 2004).

Consumption of milk fortified with multi-micronutrients reduced the incidence of pneumonia in Indian children, compared to children who received the same milk without fortification (Sazawal *et al*. 2007). Conversely, multi-micronutrient supplementation had no effect on the incidence of pneumonia in either HIV-infected or uninfected South African children (Luabeya *et al.* 2007).

Multi-micronutrient supplementation seems to improve growth in stunted children, and may be of benefit in improving appetite and reducing diarrhoea and respiratory infections in children. Investigating the impact of multi-micronutrient supplementation in HIV-infected children in relation to these outcomes would be worthwhile. In view of the various effects of micronutrients, a multi-micronutrient supplement is likely to be more beneficial in HIV-infected children than a single micronutrient. Such a supplement should at least include the following micronutrients; vitamins A, B2, B12, D and E, and iron and zinc. Deficiencies of these micronutrients are often usually associated with a diet that is low in animal source foods (Allen *et al.* 2009). In the long-term it is probably better to correct micronutrient deficiencies through an improved diet rather than supplementation with tablets. However, animal source foods tend to be expensive and are thus not easily affordable by poor communities. Therefore supplementation may be the more practical option in the short to medium-term. This is especially so when one notes that HIV-infected children are often seen at health care centres at regular intervals and the supplements can thus be easily distributed from these centres.

Public health importance of multi-micronutrient supplementation in HIV-infected children

Supplementation with micronutrients is likely to reduce the duration of and number of episodes of diarrhoea and respiratory tract infections in HIV-infected children. Supplementation might also improve the appetite and growth of these children. When one considers the fact that the most common complications seen in HIV-infected children are poor growth and undernutrition, respiratory tract infections and diarrhoea, it becomes clear that an intervention that attenuates these problems is highly relevant.

It is recognised that the mainstay of treatment in HIV-infected children is anti-retroviral therapy. In South Africa, antiretroviral therapy (ART) was generally not available to patients in the public sector (which serves the overwhelming majority of South Africans) when the present studies were conceived. The South African government made a decision to provide ART in the public sector during the latter period of 2003 and the treatment began in April 2004. However, in spite of this a large number of HIV-infected children in sub-Saharan Africa are still not on ART. South Africa has the largest (in absolute numbers) ART

programme in Africa. Despite this fact, it was estimated that the proportion of HIV-infected children on ART in South Africa was 10% in 2007 (UNAIDS, 2008). By the end of November 2009 there were 86 270 children on ART in South Africa, out of the 280 000 HIV-infected children at the same time (UNAIDS, 2010).

Therefore even in the era of expansion of the ART programme, multi-micronutrient supplementation remains an important part of the management of HIV-infected children.

Objectives of studies reported in this thesis

As outlined above HIV-infected children have a poor nutritional status as indicated by anthropometric indices and micronutrient levels. These children are also prone to diarrhoeal disease and acute respiratory infections. Micronutrient deficiencies on their own may cause immune suppression and also increase the risk of diarrhoea and respiratory infections. Children with micronutrient deficiencies are also more likely to have poor growth and poor appetite.

The main objective of the research presented in this thesis was to study whether micronutrient supplementation improves growth performance, morbidity (specifically diarrhoea and acute respiratory infections) and appetite in HIV-infected young South African children.

We started by performing a cross-sectional study where we compared the nutritional status, duration of hospitalisation and appetite of HIV-infected children who were admitted to hospital with diarrhoea and pneumonia and that of HIV-uninfected children admitted with the same diagnoses. This was done to confirm that HIV-infected children would have a poorer appetite and nutritional status and that their duration of hospitalization would be longer. The outcomes of the cross-sectional study suggested that these two groups of children were different; hence assessing them separately might be appropriate. However, there was a reasonable number of trials that have assessed the effects of multi-micronutrients in HIV-uninfected children. As previously stated, ART had recently been introduced in South African public hospitals when the study was performed (thus there were few children on ART). We thus decided to assess the short-term and long-term (over 6 months) effects of multi-micronutrient supplementation only in HIV-infected children who were not yet on ART.
We thus formulated the following hypotheses.

Short-term multi-micronutrient supplementation would reduce the duration of an acute episode of pneumonia or diarrhoea in HIV-infected children.

Long-term multi-micronutrient supplementation would improve the growth performance and reduce the number of episodes of diarrhoea or pneumonia in HIV-infected children.

A multi-micronutrient supplement over a long-term would improve the appetite of HIV-infected children.

Outline of thesis

In Chapter 2 the nutritional status, appetite and duration of hospitalisation of HIV-infected young children is compared with that of uninfected children. Chapter 3 focuses on the effect of short-term multi-micronutrient supplementation on the duration of hospitalisation in HIV-infected young children hospitalised with diarrhoea or pneumonia. In Chapter 4 we assessed whether long-term daily multi-micronutrient supplementation would improve the growth performance and reduce the number of episodes of diarrhoea or pneumonia in HIV-infected young children. Chapter 5 describes the effect of long-term multi-micronutrient supplementation on the appetite of HIV-infected young children and the levels of appetite regulating hormones. Finally, in Chapter 6 the main findings of this dissertation, nutritional aspects of children on ART and suggestions for future research are discussed.

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Chapter 2

Duration of hospitalization and appetite of HIV-infected South African children

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Chapter 2

Abstract

Human immunodeficiency virus (HIV)-infected children generally show poor growth. Episodes of diarrhoea and pneumonia in HIV-infected children are thought to be more severe than in HIV-uninfected children. The objective of this study was to compare duration of hospitalization, appetite and nutritional status of HIV-infected children with that of uninfected children. A cross-sectional study was performed on children (2-24 months) admitted with diarrhoea or pneumonia to the university hospital. Children were tested for HIV, and the duration of hospitalization was noted for 192 children. Follow-up for blood analysis (n = 154) and appetite measurement (n = 50) was performed 4-8 weeks after discharge. Appetite was measured as *ad libitum* intake of a commercial infant cereal using highly standardized procedures**.** Hospitalization (in days) was significantly longer in HIV-infected children; among children admitted with diarrhoea (5.9 ± 1.9 vs 3.8 ± 1.5) (mean ± standard deviation) and with pneumonia (9.3 ± 3.0 vs 6.1 ± 2.1). Serum zinc, iron and transferrin concentrations, and haemoglobin levels were significantly lower in HIV-infected children compared with uninfected children. Appetites [amounts eaten (g) per kg body weight] of HIV-infected children were significantly poorer than those of HIV-uninfected children (18.6 ± 5.8 vs 25.2 ± 7.4). The eating rates (g min^{-1}) of HIV-infected children were also slower (17.6 ± 6.2) vs 10.1 ± 3.7). Mean Z-scores for length-for-age were significantly lower among HIV-infected children compared to HIV-uninfected children. Weight-for-length Z-scores were not significantly different. In summary, HIV-infected children had a 52% longer duration of hospitalization and a 26% lower appetite.

INTRODUCTION

It is estimated that in 2006 there were 36 million people infected with the human immunodeficiency virus (HIV) worldwide; 24 million of these infected people were in Sub-Saharan Africa (UNAIDS 2007). The number of HIV-infected persons in South Africa is estimated at 5.4 million, of whom approximately 294 000 are children under 15 years (Dorrington *et al*. 2006). Infection with HIV has had a major impact on the admission of children to South African hospitals (Zwi *et al*. 2000). In one of South Africa's academic hospitals, approximately 60% of children admitted were HIV-infected (Pillay *et al*. 2001). The commonest admission diagnosis among HIV-infected South African children is reported to be pneumonia, followed by diarrhoea (van Deventer *et al*. 2005).

The duration of hospitalization may be longer in HIV-infected children compared with uninfected children. An evaluation of a database that accounted for approximately 73% of all US hospital discharges of children in 2000 indicated that HIV-infected children had a significantly longer mean duration of hospitalization (7.8 days vs 3.9 days), when compared with HIV-uninfected children (Kourtis *et al*. 2006). In a group of children who were younger than 5 years and were admitted to a tertiary hospital in Johannesburg, South Africa, those who are HIV-infected had an average 33% longer hospital stay than uninfected children (Meyers *et al*. 2000). HIV-infected children, who were admitted to a hospital in Cape Town, South Africa, with pneumonia had an average 33% longer duration of hospitalization when compared with HIV-uninfected children (Zar *et al*. 2001). In yet another group of children who were admitted with diarrhoea to a hospital in Johannesburg, South Africa, it was noted that the median duration of hospital stay was 8 days for HIV-infected children, as opposed to 3 days for HIV-uninfected children (Johnson *et al*. 2000). HIV-infected children from Durban, South Africa had a slightly longer duration of hospitalization than uninfected children; the difference approached but did not reach statistical significance (Pillay *et al*. 2001).

Persistent anorexia (loss of appetite) has been reported to be common among HIV-infected children (Heikens *et al*. 2008). In fact, loss of appetite was reported to be a common symptom in a group of HIV-infected Indian children, being reported in 59% of these children (Pol *et al*. 2007). Reduced energy intake is known to be common among HIV-infected people.

Indeed, a study conducted in England on HIV-infected men concluded that reduced energy intake was the prime determinant of weight in HIV associated wasting (Macallan *et al*. 1995). Loss of appetite is an important cause of reduced food intake. Oropharyngeal and oesophageal candidiasis are common with HIV infection and may lead to dysphagia (difficulty in swallowing) and odynophagia (painful swallowing) (Semba & Tang 1999). These problems also contribute to the poor food intake that occurs in these children.

The nutritional status in terms of weight, height and micronutrient status of HIV-infected children is reported to be poor compared with uninfected children. A study that followed up a group of children born to HIV-infected mothers in 11 centres in eight Europeans countries showed that between 6 and 12 months of age, uninfected children grew an estimated 1.6% and 6.2% faster in height and weight respectively, than infected children (The European Collaborative Study 2003). In Rwanda, where children born to HIV 1-infected and uninfected women were followed for 36 months, it was noted that the mean Z-scores for weight-for-age and height-for-age were lower among HIV-infected children compared with uninfected children (Lepage *et al*. 1996). The assertion that HIV-infected children have a poor micronutrient status is supported by a study that showed that approximately 60% of stable (antiretroviral naïve) HIV-infected children from Cape Town, South Africa, had multiple micronutrient deficiencies (Eley *et al*. 2002a). HIV-infected adults from the Free State province in South Africa were shown to be deficient in several micronutrients (van Staden *et al*. 1998).

Micronutrients are thought to play a role in infectious diseases, in particular diarrhoea and pneumonia. They are also thought to be important in growth and appetite. Deficiencies of zinc and vitamin A are associated with increased risk of diarrhoea and respiratory infections (Bloem *et al*. 1990; Bahl *et al*. 1998). Micronutrient deficiencies, in particular of zinc, have been associated with stunting and poor appetite (Umeta *et al*. 2000). Iron deficiency is also associated with poor appetite (Lawless *et al*. 1994).

HIV-infected children are thus more likely to have longer hospital stays and poorer appetites than uninfected children. Children with HIV infection are also likely to have poor growth and micronutrient status. The increased duration of hospitalization and poor appetites may be influenced by the poor nutritional status that is thought to be common in these children.

Antiretroviral therapy (ARV) was not available in South African public hospitals when the study was designed, and even at the conclusion of the study, only a modest number of patients were on ARVs. However, there is evidence that ARVs have an influence on the duration of hospitalization of HIV-infected children. This is supported by a retrospective study that was conducted in Italy that showed that the duration of hospitalization was longer in HIV-infected children who did not receive ARV compared to those who received the therapy (Resino *et al*. 2006). It was therefore decided to exclude children who were on ARVs.

The objective of the study was to compare duration of hospitalization, appetite and nutritional status of HIV-infected children with that of uninfected children.

SUBJECTS AND METHODS

Subjects and study area

The study was conducted between August 2004 and March 2005. The subjects were children aged 2 months-2 years who were admitted with diarrhoea and/or pneumonia to the Dr George Mukhari hospital (previously GaRankuwa hospital), the teaching hospital for the Medunsa campus of the University of Limpopo. This government owned peri-urban hospital is about 35 km north-west of Pretoria, the capital city of South Africa. The subjects were mainly from the townships surrounding the hospital. More than 80% of the patients who visit the hospital have no medical insurance and attend state-owned clinics and hospitals. Children under 6 years of age are not required to pay for hospital visits. Children admitted with acute lower respiratory tract infection (pneumonia) or acute diarrhoea were recruited to take part in the study.

Exclusion criteria

All children who had received vitamin or mineral supplements in the past 2 months were excluded. As we were assessing the length of hospitalization in children with acute pneumonia or diarrhoea, it was decided to exclude children whose pneumonia or diarrhoeal episode was longer than 72 h on admission. Children with pneumonia and respiratory failure, i.e. hypoxia on supplemental oxygen, were not included. Children who had both diarrhoea and pneumonia were also excluded. Any child who was on ARV was not eligible for inclusion.

Study design

A cross sectional study was performed. The subjects were enrolled on admission or within 24 h of admission. At enrolment, the following information was obtained: age, gender, body weight and length. The HIV status of all the subjects was determined (after counselling and informed consent) using the enzyme linked immunosorbent assay (ELISA) method in children older than 18 months, and by polymerase chain reaction (PCR) (Gene Amp 2400, Applied Biosystems, Foster City, CA, USA) in those younger than 18 months who had a positive ELISA. Children who had a documented previous positive HIV test result were not retested.

It was scheduled to enrol a total of 200 children i.e. 100 HIV-infected and 100 HIV uninfected. The treatment was as per standard hospital care, as determined by the attending physician. Children with diarrhoea were assessed for dehydration, and the dehydration was corrected over 24 h if there was hypernatraemia, or if the age was 3 months or less; otherwise rehydration was performed over 6 h. Diarrhoea was defined as the passing of three or more loose stools per day (Al-Sonboli *et al.* 2003), and pneumonia as a period during which a child was reported to have a cough, had a temperature of 38 C or more, and had an elevated respiratory rate, above the age-specific value on a minute estimation (World Health Organization 1990). The children admitted with diarrhoea were considered to ready for discharge when they had passed stools of normal consistency or no stools in the past 24 h and were well hydrated. Children with pneumonia were also considered for discharge when the temperature had been normal for at least 24 h, their respiratory rate already normal (Beatty 1998), there was no intercostal or sternal recession, and when the child is already able to drink and feed. On discharge, the duration of hospital stay was noted (from admission date to date of discharge), and the caregiver of the child was given a follow-up calendar date, written on an appointment card. The follow-up date was 4-8 weeks post discharge. At follow-up, the weight was measured; the children were assessed clinically for acute illness, by the investigator. Blood samples for serum zinc, iron, and ferritin and transferrin were taken in those who were not acutely ill. Children who were acutely ill were given another return date. All the caregivers of children who were 6 months or older (and were already consuming solids) were asked to participate with their children in the appetite-testing segment of the study, which required three additional follow-up days.

Anthropometry

The ages of the children were calculated in months by using their reported dates of birth.

The weight was measured using a single beam balance scale, without the child's shoes on and with the child wearing only light clothing, to the nearest 0.1 kg. The scale was calibrated to zero before each measurement session. The children were not clinically dehydrated at the time of the weight measurements. The length was measured in the recumbent position to 0.1 cm, on a baby board, by the investigator with the help of an assistant. One examiner held the child's head (with the chin in the neutral position) in contact with the fixed part of the board, while the other examiner stretched the child to maximum length and then brought the movable part of the board into contact with the heels. Z-scores for weight-for-age, length-for-age, and weight-for-length were calculated based on the National Centre for Health Statistics by means of the Epi-Info software version 3.2.2 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) (Dean *et al.* 2000).

Blood sampling and analysis

Blood samples were collected by venepuncture (puncture site cleaned with trace element-free alcohol). The samples for zinc were collected in trace element-free tubes with removable lids. All the blood samples were collected by the investigator and were sent to the laboratory immediately after collection, protected from light and stored at -20 C after centrifugation, until analysis. All the blood samples were stored at the Medunsa branch of the National Health Laboratory Services (NHLS) and analysed within 3 months of collection.

Serum zinc was measured by atomic absorption spectrometry in $\mu mol\ L^{-1}$ (Perkin Elmer ICP/5500, Perkin Elmer Life and Analytical Sciences Inc., Waltham, Massachusetts, USA); separation from cells was conducted within 45 min. Serum iron was measured in $\mu mol\ L^{-1}$ (SYNCHRON CX Systems IRON/TIBC Calibrator Kit, Beckman Instruments, Johannesburg, South Africa) by using rate spectrophotometry. Serum ferritin was measured using commercial ELISA kits in $\mu g\ L^{-1}$ (Access Ferritin assay, Access Immunoassay Systems, Beckman Coulter, Johannesburg, South Africa). Quality control for serum zinc, iron and ferritin was assessed by repeat analysis of standard reference material for low, normal and high values. Coefficients of variation of less than 5% were considered acceptable. Serum transferrin was measured in $\mu g\ L^{-1}$ using spectrophotometry (Beckman Immage Immunochemistry Systems and Beckman Calibrator 1, Beckman Instruments, Johannesburg,

South Africa). The C-reactive protein (CRP) was measured by nephelometry using an international reference standard for plasma proteins. CRP levels greater than 10 mg L^{-1} were considered to be elevated. Haemoglobin was analysed on whole blood by means of an electronic coulter counter in grams per decilitre.

Appetite testing

Conditions prior to testing

The subjects were given appointments on three non-consecutive days within a period of 2 weeks. The transport costs of the children and their caregivers were reimbursed on arrival at the hospital for appetite testing. From the last meal of the day preceding the appetite test, the child was not allowed to eat any food, not even breast or formula milk, until after the appetite test (Dossa *et al*. 2002). The test was conducted early in the morning. The mothers were asked when the last meal of the child was. If for any reason the child had been fed prior to the appetite test, the mother was given another date for the appetite test.

Test food

After interviewing the mothers, it was clear that the majority of the children consumed commercially manufactured infant cereals on a regular basis. The cereal Nestlé Nestum No 2 [Nestlé (South Africa), South Africa was used. For each participant, 25 g of dry porridge was weighed on an electronic load cell scale to the nearest 0.1 g. The cereal contains wheat and honey. Each 100 g of the dry cereal contains 9.3 g protein, 77.9 g carbohydrates, 1.7 g fat, and 5.2 g fibre and has 1634 kJ of energy. Fresh milk was warmed to 50 C (milk container put in a bowl of boiled water, until the milk temperature reached 50 C, measured by using a metal stem thermometer), and 160ml of this milk was added to the dry porridge and mixed to a uniform consistency. One hundred millilitres of the milk contains 3.2 g protein, 4.8 g carbohydrates, and 3.8 g fat and yields 260 kJ. The preparation of the test cereal was standardized.

Test-feeding procedures

The weight of the wet cereal was measured. An empty porcelain bowl was weighed and then the bowl with the cereal was weighed. The test was conducted in a quiet room, with three to five mother-and-child pairs at a time. Each pair sat apart from the next, and the mothers were not allowed to communicate with each other during the test. Plastic teaspoons were used for feeding the children. The mothers were informed that the child was not obliged to finish the

test food and could stop eating at anytime. The mother helped her child such that the child was eating *ad libitum*; the mother was not allowed to verbally encourage the child to eat, nor apply any kind of pressure on the child. The child was not allowed to drink water or breast milk during the appetite-testing procedure. When a child was about to complete eating, a second bowl was prepared in the same manner, and this was offered to the child to eat. When the child stopped eating, the amount eaten and the duration of the eating episode was noted. A 5-min break was then given, after which the child was invited to continue eating. The total amount eaten and the total eating duration of the eating episode were noted. The investigator supervised the feeding episodes to ensure compliance. The eating environment and utensils for each mother and child pair were standard.

Ethical considerations

The Medunsa Research Ethics and Publications Committee approved the study; the permission of the Dr George Mukhari hospital authorities was obtained, and the parents provided signed informed consent.

Statistical analysis

Anthropometric parameters, biochemical indices, duration of hospital stay and appetite test measurements were studied by means of analysis of variance (ANOVA). Among the children admitted with pneumonia there was interaction between the variables "HIV status" and "admission diagnosis" in relation to "age". Consequently, the ages of HIV-infected children with pneumonia were compared to uninfected children with pneumonia by using independent-samples *t*-test.

Correlation between weight-for-length Z-scores and chronological age, and between serum ferritin and CRP were assessed by the Pearson correlation coefficient. Correlation between anthropometric values, serum zinc and iron concentrations, and duration of hospitalization and amount eaten per kilogram body weight were also assessed using the same method (Pearson). All statistical tests were two tailed, and *P*-values less than 0.05 were considered statistically significant.

All data analyses were performed using SPSS (SPSS Inc., Chicago, Illinois, USA) statistical package for Windows version 11.0.

Chapter 2

RESULTS

In total 210 children were enrolled, but 18 refused consent for HIV testing, leaving 192 (106 HIV-infected and 86 HIV-uninfected) children. Of the 106 HIV-infected, 71 were admitted with pneumonia and 35 with diarrhoea (see Table 1). The proportion of children admitted with pneumonia was 67%, and it was 33% for those admitted with diarrhoea. Similarly, two-thirds of the HIV-infected children had pneumonia as the admission diagnosis. The overall male to female ratio was 9:10. This ratio was independent of whether the children were HIV-infected group or not and whether they were admitted with diarrhoea or with pneumonia. Of the full study population, 69% were younger than 1 year of age. ANOVA revealed a significant interaction between HIV status and admission diagnosis in relation to the subjects ages. Age was therefore used as a covariant in the ANOVA. It appeared that among children admitted with pneumonia, the mean age was significantly higher in those who were HIV-infected compared with the non-infected group (Table 1). The children were followed up 4-8 weeks after discharge for blood sampling and analysis, and the number of the children in the four subgroups is shown in Table 2. The data of appetite measurements was available for 50 children (Table 3).

Anthropometry

The anthropometric indices of the subjects are shown in Table 1. ANOVA showed that for all the anthropometric measurements, there was no significant interaction between HIV infection and admission diagnosis. Weight-for-age and length-for-age Z-scores were significantly lower among HIV-infected children compared with HIV-uninfected children. The admission diagnosis (i.e. pneumonia or diarrhoea) did not seem to significantly affect weight-for-age or length-for-age Z-scores. There was no significant difference in the weight-for-length Z-scores between HIV-infected and HIV-uninfected children. However, ANOVA showed a significant interaction between the subjects ages and weight -for-length Z-scores. Pearson's correlation coefficient showed a significant negative correlation between age and weight-for-length Z-scores ($P < 0.001$).

Table 1. Anthropometric characteristics and duration of hospitalization of all the study children

	Pneumonia		Diarrhoea		All HIV-	All HIV-
	HIV-uninfected	HIV-infected	HIV-uninfected	HIV-infected	uninfected	infected
n	58	71	28	35	86	106
Gender (M/F)	35/23	30/41	10/18	16/19	45/41	46/60
Age (months)	7.4 ± 5.7*	11.1 ± 6.4	11.4 ± 7.6	9.1 ± 6.8	8.7 ± 6.3	10.4 ± 6.5
WAZ	-1.02 ± 0.97*	-1.88 ± 0.83	-0.89 ± 1.02†	-1.46 ± 0.76	-0.97 ± 0.99*	-1.74 ± 1.20
HAZ	-0.65 ± 1.31*	-1.72 ± 0.85	-0.71 ± 1.10*	-1.62 ± 0.88	-0.67 ± 1.24*	-1.68 ± 0.86
WHZ	-0.61 ± 1.11	-0.78 ± 0.88	-0.59 ± 1.12	-0.46 ± 0.95	-0.60 ± 1.11	-0.67 ± 0.90
Duration (days)	6.1 ± 2.1*	9.3 ± 3.0	3.8 ± 1.5*	5.9 ± 1.9	5.4 ± 1.9*	8.2 ± 2.6

HIV, human immunodeficiency virus; WAZ, weight -for-age Z-score; HAZ, length-for -age Z-score; WHZ, weight-for-length Z-score. Mean ± standard deviation (n). *Significantly different from HIV-infected group (P <0.01). †Significantly different from HIV-infected group (P <0.05)

Duration of hospitalization

Table 1 also provides the results of the comparison of the duration of hospitalization of HIV-infected children with that of uninfected children. The duration of hospitalization was 52% longer (9.3 days compared with 6.1 days) in HIV-infected children admitted with pneumonia as compared with non-HIV-infected children with pneumonia; this difference was significant (P < 0.001). Among children admitted with diarrhoea, the duration of hospital stay was 55% longer (5.9 days compared with 3.8 days) in HIV-infected children (P <0.001). Children admitted with pneumonia had a significantly longer hospital stay (53% longer) compared with those admitted with diarrhoea (P < 0.001). The Pearson correlation coefficient also revealed a significant negative correlation between serum zinc concentrations and duration of hospitalization, and between weight-for-age Z-scores (WAZ), length-for-age Z-scores (HAZ) and duration of hospitalization.

Table 2. Biochemical indices 4 to 8 weeks after hospital discharge

	Pneumonia		Diarrhoea	
	HIV-uninfected	HIV-infected	HIV-uninfected	HIV-infected
Serum zinc (μmol L^{-1})	5.8 ± 2.9 (48)*	4.7 ± 2.8 (50)	67 ± 3.1 (24)*	5.3 ± 2.9 (28)
Serum iron (μmol L^{-1})	7.0 ± 4.3 (48)†	5.2 ± 3.23 (51)	7.5 ± 4.4 (24) †	6.5 ± 4.6 (28)
Serum ferritin (μg L^{-1})	76.5 ± 72.9 (43)	90.3 ± 83.8(44)	67.3 ± 54.6 (25)*	143.0 ± 105.0 (26)
Transferrin (g L^{-1})	2.4 ± 0.9 (43)*	2.1 ± 1.1 (41)	2.7 ± 0.9 (19)*	2.1 ± 1.0 (25)
Haemoglobin (g dL^{-1})	10.8 ± 1.9 (50)*	9.5 ± 2.1 (47)	10.5 ± 2.0 (25)*	9.9 ± 2.0 (28)
CRP >10 mg L^{-1} (%)	26% (26)*	35% (35)	12% (12) *	19% (19)

HIV, human immunodeficiency virus ; CRP, C -reactive protein. Mean ± standard deviation (n). *Significantly different from HIV -infected group (P <0.01), †Significantly different from HIV-infected group (P = 0.05)

Biochemical indices

The biochemical indices of the subjects are shown in Table 2. ANOVA showed no significant interaction between HIV status and admission diagnosis with respect to the concentrations of serum zinc, iron and transferrin, and the haemoglobin levels. Serum zinc, iron and transferrin concentrations, and haemoglobin levels were significantly lower in HIV-infected children compared with the uninfected children. ANOVA revealed a significant interaction between HIV status and admission diagnosis in relation to serum ferritin concentrations. Independent samples *t*-test done subsequently showed no significant difference in serum ferritin concentrations of HIV-infected and non-HIV-infected children with pneumonia; however, HIV-infected children with diarrhoea had significantly higher serum ferritin concentrations compared with non-infected children with diarrhoea. A large proportion of both HIV -infected and non-infected children were anaemic (Hb < 11g d L^{-1}), 75% in HIV-infected children and 58% in HIV-uninfected children. ANOVA revealed that HIV -infected children had significantly higher CRP levels independent of admission diagnosis (P < 0.05). The percentage of children with CRP levels less than 10mg L^{-1} among HIV-infected and HIV-uninfected children was 30% and 21% respectively. Pearson's correlation coefficient showed a significant positive correlation between serum ferritin and CRP levels (P < 0.01).

Table 3. Appetite test 4 to 8 weeks after hospital discharge

	Pneumonia		Diarrhoea		All HIV-	All HIV-
	HIV-uninfected	HIV-infected	HIV-uninfected	HIV-infected	uninfected	infected
n	13	17	11	9	24	26
Gender (M/F)	6/7	6/11	3/8	5/4	9/15	11/15
Age (months)	11.9 ± 4.1	14.1 ± 5.3	14.2 ± 6.3	12.0 ± 4.2	13.0 ± 5.1	13.4 ± 4.9
Weight (kg)	8.6 ± 1.8	7.9 ± 1.4	9.2 ± 1.7	8.1 ± 1.0	8.9 ± 1.8*	8.0 ± 1.3
Amount eaten (g)	190 ± 53*	153 ± 54	230 ± 99†	148 ± 47	208 ± 74*	148 ± 47
Amount eaten (g kg^{-1} Bwt)	21.8 ± 7.1	18.8 ± 6.2	29.3 ± 7.8 *	18.1 ± 4.9	25.2 ± 7.4*	18.6 ± 5.8
Duration of eating (min)	12.1 ± 3.2 *	15.6 ± 3.0	14.9 ± 4.7	15.6 ± 3.1	13.4 ± 3.9†	15.6 ± 3.0
Eating rate (g min^{-1})	16.5 ± 5.0*	10.3 ± 3.8	18.8 ± 7.7*	9.8 ± 3.4	17.6 ± 6.2*	10.1 ± 3.7

HIV, human immunodeficiency virus. Mean ± standard deviation (n). *Significantly different from HIV-infected group (P <0.01). †Significantly different from HIV-infected group (P =0.05).

Appetite test

The total intake of the test food eaten, the amount of test food per kilogram body weight, the eating duration and the eating rates are shown in Table 3. The amount of test food eaten was significantly lower in HIV-infected children compared with those who were uninfected. The duration of eating was significantly longer in children who were HIV-infected than in the uninfected ones. ANOVA revealed that the amount of test food eaten per kilogram was 2 6% lower (statistically significant), and the eating rate was significantly lower in HIV-infected children compared with uninfected children, independent of the admission diagnosis. The admission diagnosis (pneumonia or diarrhoea) did not significantly affect the eating duration, eating rate or the amount eaten per kilogram body weight. The Pearson correlation coefficient also revealed a negative correlation between serum zinc concentrations and amount eaten per kilogram body weight, and between WAZ, HAZ and amount eaten per kilogram body weight, but these were not statistically significant.

Chapter 2

DISCUSSION

The objective of this study was to study the duration of hospital stay, appetites and nutritional status of HIV-infected South African children compared with uninfected children. The length of hospital stay was significantly longer in children infected with HIV compared with those who were not infected. The appetites of HIV-infected children were significantly poorer than the non-infected children. The children with HIV infection were significantly more stunted, but not more wasted, compared with the uninfected ones. The micronutrient status of children infected with HIV was poor compared to those who were uninfected.

Considering the objectives of the study, it was important to adequately define HIV-infected children in distinction to HIV-uninfected children, and this was done by the ELISA test and PCR as described in the Subjects and Methods section. However, within the group of children who were HIV-infected, clinical and immunological staging of the HIV disease was not done. It is recognised that the outcome of the study could have been affected by the stage of HIV.

Approximately 55% of the study children were HIV-infected; this is in keeping with the proportions observed at the hospital. There were more females than males among the subjects, and in the HIV-infected group of children, the proportion of females was also higher. However, there was no significant association between the gender of the child and whether the child was HIV-infected or not. There was also no association between the gender of the children and whether they were admitted with diarrhoea or pneumonia, therefore the results of boys and girls were combined. HIV-infected children admitted with pneumonia were significantly older than the uninfected children. This is thought to be as a result of the fact that diarrhoea and pneumonia occur more commonly in infants than in older children (Kosek *et al*. 2003; Zar *et al*. 2005). However, HIV-infected children remain vulnerable to these diseases for a longer period than the uninfected children, such that HIV-infected children with pneumonia or diarrhoea may on average be older than uninfected children. This may explain the age difference that we noted in the current study. Certainly, other authors have made a similar observation; a study performed on Ugandan children younger than 18 months admitted with sepsis noted that the median age of HIV-infected subjects was higher than that of uninfected subjects (Bakaki *et al*. 2001). This was also noted in a group of HIV-infected

children (aged 2 months-5 years) admitted with severe lower respiratory tract infection to a hospital in Johannesburg, South Africa, where the median age of HIV-infected children was significantly higher than that of uninfected children (Madhi *et al*. 2000a).

The duration of hospital stay was approximately 53% longer among HIV-infected children, compared to uninfected ones. Among children admitted with diarrhoea those with HIV infection had a 55% longer hospital stay. Similarly, in the group of children admitted with pneumonia it was 53% longer. The difference in duration of hospitalization of 2-3 days is also considered clinically significant. The longer duration of hospitalization among HIV-infected children is consistent with the findings of a number of studies. A study conducted in the United States which evaluated a database that accounted for approximately 73% of all US hospital discharges of children in 2000, revealed that HIV-infected children had longer mean hospital stays (7.8 days versus 3.9 days), when compared with HIV-uninfected children (Kourtis *et al*. 2006). South African studies have reported similar results. In Johannesburg, HIV-infected children admitted to a tertiary hospital had a 33% longer hospital stay (Meyers *et al*. 2000). Likewise, HIV-infected who were admitted to hospitals in Cape Town and Johannesburg with lower respiratory tract infection had a longer duration of hospitalization than HIV-uninfected children (7-14 days compared with 6-10 days) (Madhi *et al*. 2000b; Zar *et al*. 2001). In the same way, the median duration of hospital stay was 8 days for HIV-infected children admitted to a hospital in Johannesburg with diarrhoea, as opposed to 3 days for HIV-uninfected children with diarrhoea (Johnson *et al*. 2000).

Children with HIV infection had significantly lower appetites compared with uninfected children in this study. This was in terms of total amount eaten, duration of eating episodes and eating rate, and was independent of the admission diagnosis. The total amount of test food eaten was 26% lower in the group of children who were HIV-infected, compared with those who were uninfected. The children who were infected with HIV also ate significantly less per kilogram body weight than the uninfected children. The amount eaten per kilogram body weight was 15%-20% lower in the HIV-infected group of children. The HIV-infected children took longer to complete eating the smaller amount of test food, and this resulted in an eating rate that was 60% slower than that of the HIV-uninfected children. The *ad libitum* consumption of the test food was used as a proxy for measuring appetite in this study. This appetite test has been validated as an appropriate tool in appetite evaluations (Dossa *et al*.

2002). However, it is recognised that there may be other reasons contributing to reduced food intake in HIV-infected children. Oro-pharyngeal candidiasis is known to be common in HIV-infected children (Pol *et al*. 2007), and this may lead to painful swallowing and thus reduced food intake. The proportion of children with oro-pharyngeal candidiasis was not assessed in this study. Reduced energy intake is thought to be common in HIV-infected children, and this reduced energy intake is thought to play a major role in the poor growth of HIV-infected people (Macallan *et al.* 1995). A study comparing HIV-infected American children with growth failure (growth velocity ≥ 5th percentile for age) with HIV-infected children without growth failure noted that the mean age-adjusted energy intake per day was significantly lower in the group of children with growth failure (Arpadi *et al*. 2000). Because of a lack of similar studies in young HIV-infected children, the results of the appetite test cannot be compared. Nonetheless, we believe that the poor appetites in HIV-infected children in our study were adequately demonstrated.

The weight-for-age and length-for-age Z-scores of the HIV-infected children were significantly poorer than that of uninfected children. However, there was no significant difference in the weight-for-length Z-scores. Our finding that length-for-age Z-scores were significantly lower among HIV-infected children compared to the non-infected children was consistent with other studies that have suggested that stunting is frequently an early finding in perinatal HIV infection. Bobat *et al*. (2001) observed a cohort of children born to HIV-infected South African women and noted that by 3 months of age, the children who were HIV-infected were significantly more stunted, but not more wasted than those who were not HIV-infected. The poor weight-for-age Z-scores and stunting that occurs in these infants may be related to poor intra uterine growth as a result of poor maternal nutrition. Nonetheless, there are post-natal causes of stunting as well, including decreased energy intake (Arpadi *et al.* 2000), nutrient malabsorption (Knox *et al.* 2000) and increased resting energy expenditure per kilogram of fat-free mass (Batterham 2005). We may assume that the weights and lengths will be similarly affected by the poor maternal nutritional status, such that wasting is not evident in the first few months of life. However, as the child's own immunological status deteriorates, wasting may then ensue. Maternal nutritional status before and during gestation is thought to be one of the strongest determinants of pregnancy outcomes (Villamor *et al*. 2002). Pregnant HIV-infected women have been observed to be at higher risk than those who

are not infected of intrauterine growth retardation and having low birth weight babies (Iroha *et al*. 2007). Poor maternal weight, advanced-stage HIV disease and intrauterine HIV transmission were identified as significant determinants of having a low birth weight baby in a group of pregnant HIV-infected Tanzanian women (Dreyfuss *et al*. 2001). The assertion that wasting is likely to be a late finding among children with HIV infection is supported by a study conducted among pregnant HIV-infected women in the Democratic Republic of Congo (DRC). In this study from the DRC, it was shown that when compared with pregnant HIV-uninfected women, the children of the HIV-infected women were significantly more stunted by 3 months of age, while they only became significantly more wasted by 12 months of age (Bailey *et al*. 1999). In our study, there was no significant difference in weight-for-length Z scores between HIV-infected and uninfected children.

In the current study, the zinc and iron status of HIV-infected children was significantly poorer than that of uninfected children. Similar findings have been observed in a number of studies. Low concentrations of plasma zinc have been noted to be highly prevalent in HIV-infected female drug users (Baum *et al*. 2003). Micronutrient deficiencies were also noted to widespread in stable HIV-infected South African children (Eley *et al*. 2002a). Anaemia has also been shown to be common in HIV-infected children; another study from Cape Town, South Africa, indicated that 72% of HIV-infected children were anaemic (Eley *et al*. 2002b). The factors that result in micronutrient deficiencies in HIV-infected subjects include the following: (1) decreased intake of micronutrients as a result of poor appetite, central nervous system disease and dysphagia; and (2) diarrhoea and malabsorption of micronutrients, and altered metabolism with increased utilization of the micronutrients. These factors have been well described in the review by Semba & Tang (1999).

Micronutrient deficiencies are known to be associated with increased severity of infectious diseases, especially diarrhoea and respiratory tract infections (Bloem *et al*. 1990; Bahl *et al*. 1998). Deficiencies of micronutrients have also been observed to be associated with poor growth. This has been elucidated by a review by Rivera *et al*. (2003), which noted that there is strong evidence for the contribution of micronutrients to growth faltering, in particular deficiencies of zinc, vitamin A and iron. The longer duration of hospitali zation observed in the current study is thought to be in part related to the poor nutritional status (anthropometry and micronutrient status) of the HIV-infected children. Indeed, we did show a

significant negative correlation between anthropometric data (WAZ and HAZ), serum zinc concentrations and the duration of hospital stay. Micronutrient deficiencies, in particular zinc deficiency, have been associated with poor appetite (Baum *et al*. 2000). Moreover, zinc supplementation has been shown to significantly improve appetite in infants (Umeta *et al*. 2000). The poor growth observed in these children is thought to be partly related to reduced energy intake, which is influenced by appetite.

Results from this study show that children with HIV infection have a longer duration of hospitalization and were noted to have poorer appetites than the control group. The nutritional status of HIV-infected children as measured by growth and micronutrient status was significantly poorer than that of non-infected children.

A holistic approach is needed in the potential interventions in HIV-infected children. Potential interventions include early introduction and scaling up of antiretroviral therapy, focusing on appetite and food intake and possibly micronutrient supplementation. Future research will indicate whether improving the dietary intake of HIV-infected children and/or supplementation with multi-micronutrients will improve the nutritional status of these children.

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Chapter 2

Chapter 3

Short-term micronutrient supplementation reduces the duration of pneumonia and diarrheal episodes in HIV-infected children

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Chapter 3

Abstract

The duration of pneumonia and of diarrhea is reported to be longer in HIV-infected children than in uninfected children. We assessed the effect of a multi-micronutrient supplement on the duration of hospitalization in HIV-infected children. In a double-blind randomized trial, HIV-infected children (4-24 mo) who were hospitalized with diarrhea or pneumonia were enrolled (n = 118) and given a daily dose of a multi-micronutrient supplement (containing vitamins A, B complex, C, D, E and folic acid, as well as copper, iron and zinc at levels based on recommended daily allowances) or a placebo until discharge from the hospital. Children's weights and heights were measured after enrollment and micronutrient concentrations were measured before discharge. On recovery from diarrhea or pneumonia, the children were discharged and the duration of hospitalization was noted. Anthropometric indices and micronutrient concentrations did not differ between children who received supplements and those who received placebos. Overall, the duration of hospitalization was significantly shorter ($P < 0.05$) among children who were receiving supplements (7.3 ± 3.9 d) (mean ± SD) than in children who were receiving placebos (9.0 ± 4.9); this was independent of admission diagnosis. In children admitted with diarrhea, the duration of hospitalization was 1.6 d (19%) shorter among children receiving supplements than in those receiving placebos, and hospitalization for pneumonia was 1.9 d (20%) shorter among children receiving supplements. Short-term multi-micronutrient supplementation significantly reduced the duration of pneumonia or diarrhea in HIV-infected children who were not yet receiving antiretroviral therapy and who remained alive during hospitalization.

INTRODUCTION

There were ~2.5 million children living with HIV worldwide in 2007 and 90% of them were in sub-Saharan Africa (1). In South Africa, there are ~300 000 children up to the age of 14 y infected with HIV (2). In sub-Saharan Africa, the HIV epidemic has resulted in a marked increase in childhood respiratory and diarrheal disease-related morbidity and mortality (3-5). In a South African academic hospital, the most common admission diagnosis among HIV-infected children was pneumonia, followed by diarrhea; these diseases were also noted to be more common in HIV-infected children than in uninfected children (6).

A survey in the USA showed that the duration of hospital stays was longer in HIV-infected children than in uninfected children (7). HIV-infected South African children hospitalized with diarrhea or pneumonia also need longer periods of hospitalization (8, 9, 6, 10). In a cross-sectional study at our hospital in Pretoria, we detected that duration of hospitalization was significantly longer (2.5 d) and that the micronutrient status was significantly poorer (our unpublished data). Micronutrient deficiencies are indeed known to be common in HIV-infected children (11, 12). The severity of the micronutrient deficiencies may be partly related to the stage of the HIV disease (13) and to the severity of the pneumonia and/or diarrheal illness (14).

Studies assessing the effect of micronutrient supplementation on the duration of episodes of diarrhea or pneumonia reveal conflicting results. Zinc supplementation was shown to be beneficial in reducing the duration of diarrhea in South Asian children (15-18) but not in Bangladeshi male infants aged 1-6 mo (19). Analyses of pooled data from trials of acute diarrhea have concluded that zinc therapy is beneficial in the treatment of acute diarrhea (20, 21). Although it has been found that zinc supplementation may reduce the duration of pneumonia (22), other studies failed to confirm this effect (23, 24). The addition of vitamin A to the zinc supplement may have a synergistic effect on reducing diarrhea (25), partly due to the fact that zinc is essential for both intra- and intercellular transport of vitamin A (26, 27). However, other studies do not show this effect (18, 16). Vitamin A supplementation was shown to significantly reduce the duration of hospitalization among Mozambican children who were admitted with non-measles acute lower respiratory infections (28). Nonetheless, a

metaanalysis revealed that there is no evidence that high-dose vitamin A improves recovery from pneumonia in developing countries (29).

Given that micronutrient supplements might be more effective in children who are malnourished (15, 30, 31), supplementation might be beneficial in children who are HIV-infected. However, studies on the effects of micronutrient supplementation on the duration of hospitalization of HIV-infected children are scarce.

Because micronutrient deficiencies rarely occur as single nutrient problems but rather as simultaneous deficiencies of more micronutrients, it is considered that the deficiencies should be addressed concurrently (32, 33). The proportion of HIV-infected South African children receiving antiretroviral therapy (ART) in 2007 was estimated to be 10% according to UNAIDS (1). We therefore performed a randomized controlled trial to test our hypothesis that a multi-micronutrient supplement would significantly reduce the duration of hospitalization in HIV-infected children admitted with acute diarrhea and pneumonia who are not receiving ART.

PARTICIPANTS AND METHODS

Participants and study area

The participants were HIV-infected children with ages between 4 mo and 2 y (overall number of children assessed for eligibility = 389; number of enrolled children = 118). These children had been admitted with diarrhea or pneumonia to the pediatric wards of Dr George Mukhari hospital, the academic hospital for the Medunsa campus of the University of Limpopo. This public hospital is the second largest in the republic of South Africa with ~1800 beds. It is located ~35 km north-west of Pretoria, the capital city of South Africa. All the children were from the townships surrounding the hospital. The study was conducted between November 2005 and May 2007.

Diarrhea was defined as the passing of ≥ 3 loose stools/d in the previous 24 h as reported by the parent or guardian. Pneumonia was defined as an illness during which a child is reported to have a cough, has a temperature of 38 C or more, and has an elevated respiratory rate above the age specific value on a minute estimation (50 breaths/min) (34). Because we

intended to assess the effects of supplements on acute diarrhea, it was decided that children whose diarrheal episode was already longer than 72 h on admission should be excluded. Children admitted with pneumonia complicated by respiratory failure, i.e. hypoxia (saturation <90% on optimum amount of supplemental oxygen) and/or hypercarbia (Pco2 > 50 mmHg), were also not included, because these children were eligible for transfer to the intensive care unit. To assess the effects of the supplements as such, children who were on ART or those who had received vitamin or micronutrient supplementation in the previous 2 mo were considered not eligible for inclusion. The proportion of HIV-infected South African children (<14 y) receiving ART in 2007 was estimated to be 10% according to UNAIDS (1). The proportion of HIV-infected children aged 4-24 mo who were on ART is not known, but it was probably below 10% (Dr M.C. Moshe, personal communication).

Children who had a chronic illness unrelated to HIV were also excluded (total number excluded = 271; 258 because of not meeting inclusion criteria and 13 because of refusing to participate).

The Medunsa Research Ethics and Publications Committee approved the study; the permission of the GaRankuwa (now Dr George Mukhari) hospital authorities was obtained. The parents or guardians of all participants provided signed informed consent prior to commencing the study.

Study design

A randomized, double-blind, placebo-controlled study was performed. A simple randomization schedule (computer-generated random numbers) was used to assign the children to 1 of 2 groups to receive a multi-micronutrient supplement or a placebo, stratified by admission diagnosis (pneumonia or diarrhea).

The HIV status of the children was confirmed by laboratory analysis. HIV-infected children whose parents agreed to participate in the study were enrolled within 24 h after admission. The assessment for eligibility (including obtaining consent for participation in the study) was conducted in the pediatric admission ward of the hospital in the morning (around breakfast time); this process lasted ~2h. The supplement was then given to children who met the inclusion criteria and were willing to participate. The timing of the subsequent daily dose of supplement or placebo was standardized and was scheduled to be given at breakfast. Thus,

the administration of supplement or placebo started on the morning of enrolment. The body weights and lengths of the children were measured when the child was fully rehydrated, usually within 24 h of enrolment.

The principal investigator monitored the progress of the children in the ward and when the attending physicians were of the opinion that the child was likely to be discharged (in line with discharge criteria) within 1 or 2 d, then blood samples were taken from fasting children for serum zinc, serum retinol, iron, and ferritin, plasma C-reactive protein (CRP), and hemoglobin (Hb) analyses. On the day of discharge, the duration of hospitalization was noted. Blood samples were also taken for CD4 and CD8 counts (within 24 h of admission) and these were used to classify the severity of HIV according to the CDC classification (35).

Treatment

The supplements and placebos used were crushable tablets provided by the pharmaceutical company Adminicle Trading. The multi-micronutrient supplements contained 300 μg retinol, 0.6 mg thiamine, 0.6 mg riboflavin, 8 mg niacin, 0.6 mg pyridoxine, 1 μg cobalamin, 70 μg folic acid, 25 mg ascorbic acid, 5 μg 1,25-dihydrocholecalciferol, 7 mg d,l α-tocopherol, 700 μg copper, 8 mg iron, 30 μg selenium and 8 mg zinc at amounts based on recommended dietary allowances for a 1-y-old child (36). The amounts in the supplement were independently confirmed and certified by the Medicines Control Council of South Africa. The placebo and supplement tablets were identical in appearance and taste; the containers were also identical and were labeled only by study serial number. The supplements or placebos were crushed using a pill crusher and mixed with water and were administered by the nurse in the ward.

Methods

Clinical evaluation. The children were all examined by the principal investigator to look for any chronic illnesses. All the children were treated according to standard hospital protocols for diarrhea and pneumonia. The children with pneumonia were all given first-line antibiotics for community-acquired pneumonia. The children were ready for discharge when they passed two consecutive formed stools or no stools passed in the previous 24 h, as reported by the nurses in the hospital records. A child was considered to have recovered from pneumonia when the temperature and respiratory rate had been normal for at least 24 h, when there was

no intercostal or sternal recession, and the child was able to drink and eat. The attending physicians discussed with the principal investigator prior to discharge to ensure adherence to the discharge criteria.

HIV tests. HIV-1 and HIV-2 serostatus was ascertained using 2 ELISA tests (Abbot Diagnostics) in children older than 15 mo (37). In children younger than 15 mo, a PCR test was performed in addition to the 2 ELISA (37). The PCR test (Gene Amp 2400, Applied Biosystems) was performed in the Virology laboratory of the National Health Laboratory Services (NHLS) at the Medunsa campus of the University of Limpopo.

Anthropometry. The ages of the children were calculated in months by using the dates of birth, as given by the mothers. The weight was measured to the nearest 0.1 kg without shoes and with the child wearing only light clothing using a single beam balance scale. The scale was calibrated before each measurement session using a standard weight of 10 kg. The children were not clinically dehydrated at the time of the weight measurements. The length was measured in the recumbent position to 0.1 cm, on a baby board, by the investigator with the help of an assistant. One examiner held the child's head (with the chin in the neutral position) in contact with the fixed part of the board while the other examiner stretched the child to maximum length and then brought the movable part of the board into contact with the heels. Z-scores for weight-for-age (WAZ), length-for-age (HAZ), and weight-for-length (WHZ) were calculated based on the National Centre for Health Statistics (NCHS) reference values by means of the Epi-Info software version 3.2.2 (38).

Blood sampling and analysis. Blood samples were collected by venipuncture (puncture site cleaned with trace element-free alcohol). The samples for zinc were collected in trace element-free tubes, with removable lids.

All blood samples were collected after an overnight fast and were sent to the laboratory immediately after collection, protected from light, and stored at -20 C after centrifugation, until analysis. All the blood samples were stored at the Medunsa branch of the NHLS.

The serum zinc, serum iron, and ferritin measurements were performed at the Medunsa branch of the NHLS, while the retinol samples were batched and sent frozen to the main branch of the NHLS in Johannesburg and were analyzed within 10 d of sampling. Serum zinc was measured by atomic absorption spectrometry (Perken Elmer ICP/550) (39), serum retinol by a fluorometric method (40), serum iron by rate spectrophotometry (39) (SynchronCX

System, Beckman Coulter), and serum ferritin by using commercial ELISA kits (Beckman Access 2, Beckman Coulter) (41). Quality control for serum zinc, iron, and ferritin was assessed by repeat analysis of standard reference material for low, normal, and high values. The source of the reference material was Bio-Rad Laboratories for serum zinc and iron and the material for the serum ferritin was obtained from Beckman Coulter. The intra-assay and inter-assay CV for serum zinc, retinol, iron, and ferritin were all < 5%.

The Hb concentration was analyzed spectrophotometrically in whole blood (ABX Pentra DX 120, Horiba ABX Diagnostics). It was measured to test for anemia, defined as Hb of less than 110 g/L (42).

Plasma concentrations of CRP were measured by nephelometry, using an international reference standard for plasma proteins. CRP levels > 10 mg/L were considered to be high, and indicative of inflammation. The CD4 lymphocyte counts were measured using a Coulter flow cytometer (Coulter Epics XL-MCL, Beckman Coulter) to assess the immunological stage of HIV infection. The CD4 were reported as CD4 T-cell percentage (percent of total T-lymphocyte that is of the CD4 lineage) rather than absolute CD4 counts, because the CD4 percentages are commonly used in clinical pediatric practice and have less age-related variability (43).

Statistical analysis

Anthropometric Z-scores were calculated with Epi-Info software (version 3.2.2). Data elaboration and analysis was performed using SPSS 13.0 for Windows. Statistical analyses were 2-tailed where appropriate and significance was set at 5%.

The sample size calculation was based on a cross-sectional study conducted at the same hospital (our unpublished data) that revealed a mean duration of hospitalization of 5.9 ± 1.9 and 9.0 ± 2.5 d for HIV-infected children admitted with diarrhea and pneumonia respectively. The calculations were based on a 5% level of significance and 80% power for a 2-tailed test, designed to be able to detect 1.5 d in the duration of diarrhea and of pneumonia. The required sample sizes per treatment group were 26 and 29 for diarrhea and pneumonia, respectively (44). In the present study, we ended up with slightly lower numbers than were calculated, but these lower numbers hardly affected the power of the tests. However, the power of the tests was strongly influenced by the much larger actual between-child duration of hospitalization

(twice as much as we used in our a priori power calculations). Therefore, the actual power with which a relevant difference of 1.5 d in duration of hospitalization could be tested between treatment and placebo groups for all children was only 40%. Nevertheless, we did observe a significant treatment effect. However, in future studies, power calculations should be based on larger between-child variation in duration of hospitalization.

Analyses were conducted using coded treatment groups with the analysts blinded to the actual treatment.

Data were assessed for normality by visual examination of distribution plots followed by normality tests, and were normalized where appropriate by log transformation.

Anthropometric measurements, serum zinc, retinol, iron, ferritin, and CD4 lymphocyte percentages and Hb concentrations, were compared using ANOVA with treatment group and gender as between-subject factors. The proportion of children with anemia and with CRP levels > 10 mg/L was compared between the 2 groups by the chi-square test.

The differences in the duration of hospitalization of the 2 treatment groups were also contrasted by means of ANOVA, with admission diagnosis as a cofactor and age and gender as covariates. The effect of a covariate on the dependent variable was used to assess the interaction effect. ANOVA with or with out the inclusion of data of children who died (i.e. intention to treat analysis) did not differ significantly from each other.

RESULTS

A total of 389 children were screened, of whom 118 children were enrolled. Out of the 118 enrolled children, 50 were admitted with diarrhea and 68 with pneumonia (**Fig. 1**). In total, 12 (10%) children died during the period of hospitalization: 4 from the diarrhea group and 8 from with pneumonia group. In relation to treatment, 7 out of the 12 children who died were from the placebo group and 5 from the supplement group. The children who died were slightly younger and had lower weights and lengths than those who completed the study, but the WAZ, HAZ and WHZ did not differ (**Supplemental Table 1**).

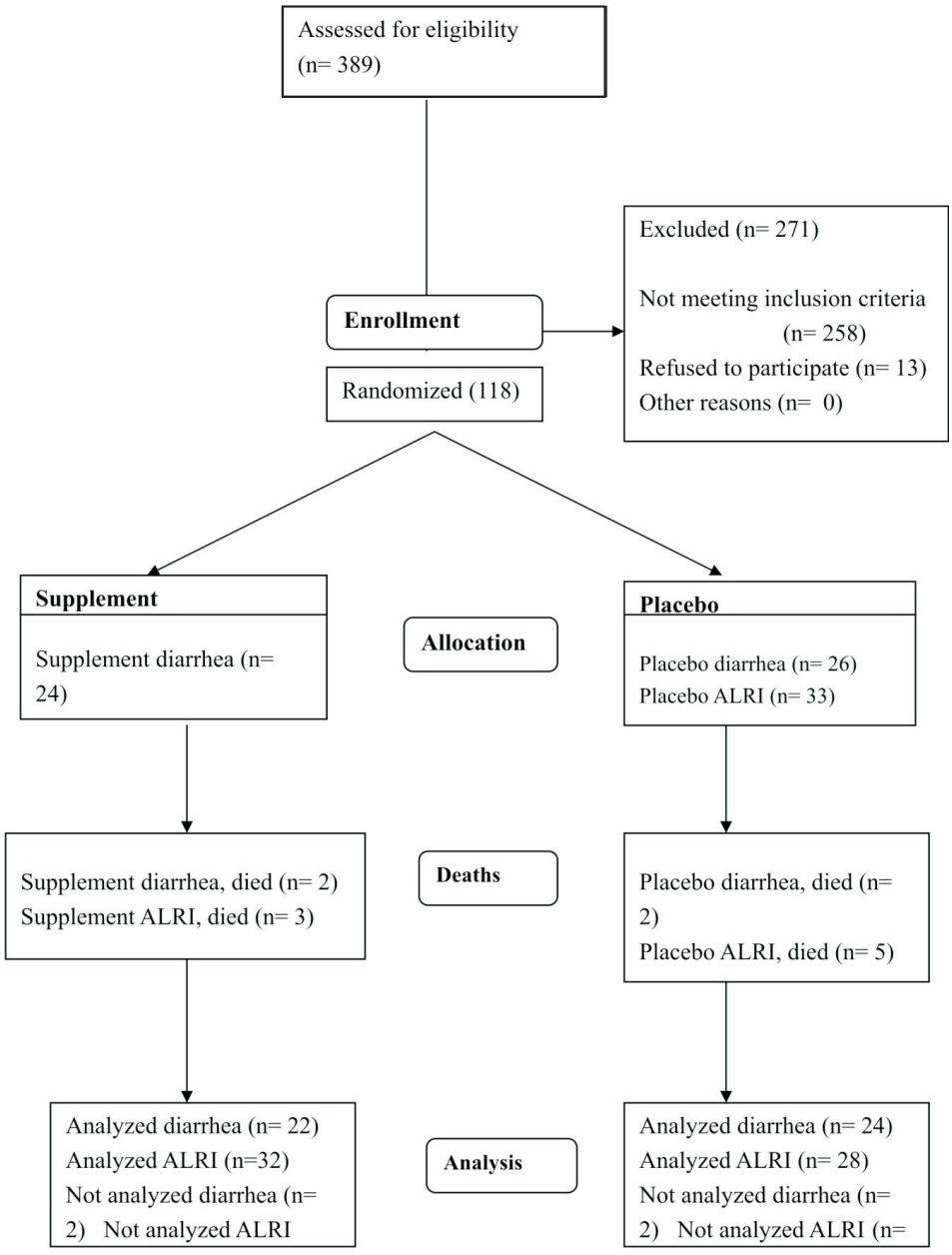

Figure 1: Profile of study subjects

Table 1. Anthropometric data of HIV-infected children with diarrhea or pneumonia administered a supplement or placebo who completed the study [1]

	Diarrhea		Pneumonia		All children who completed	
	Placebo	Supplement	Placebo	Supplement	Placebo	Supplement
n	24	22	28	32	52	54
Male/Female	11/13	11/11	14/14	20/12	25/27	31/23
Age (months)	12.4 ± 5.3	14.2 ± 6.7	9.0 ± 3.6	12.5 ± 5.7#	10.6 ± 4.8	13.2 ± 6.1#
Weight (kg)	6.6 ± 1.4	6.8 ± 1.7	5.8 ± 1.6	6.8 ± 1.6#	6.2 ± 1.5	6.8 ± 1.6#
Height (cm)	68.2 ± 7.1	69.5 ± 7.4	64.4 ± 7.1	68.4 ± 6.9#	66.1 ± 7.3	68.8 ± 7.1
WAZ	-2.71 ± 1.31	-2.83 ± 1.16	-2.83 ± 1.24	-2.57 ± 1.43	-2.77 ± 1.26	-2.68 ± 1.32
HAZ	-2.34 ± 1.59	-2.42 ± 1.72	-2.31 ± 1.62	-2.22 ± 1.55	-2.32 ± 1.59	-2.30 ± 1.61
WHZ	-1.63 ± 1.41	-1.73 ± 1.38	-1.45 ± 1.21	-1.52 ± 1.34	-1.53 ± 1.30	-1.61 ± 1.35

[1]Values are the mean ± SD. #Different from placebo group, $P < 0.05$.

The male:female ratio was similar among the children randomized into the 2 treatment groups as well as for children admitted with diarrhea and with pneumonia. Among children admitted with pneumonia, those who were receiving the supplement were older than those receiving the placebo ($P < 0.05$). However, among those admitted with diarrhea age did not differ. WAZ, HAZ, and WHZ of the children did not differ between treatment groups, or between the children admitted with diarrhea or pneumonia (**Table 1**).

The serum zinc, vitamin A, iron, and ferritin concentrations and the CD4 lymphocyte percentages did not differ significantly between the 2 treatment groups and did not differ by diagnosis at admission (**Supplemental Table 2**). With respect to Hb levels, we found a significant interaction between admission diagnosis and the age of the children, but there was no treatment effect. The mean Hb levels were lower in the group of children admitted with pneumonia (89 ± 18 g/L) than those in children admitted with diarrhea, (100 ± 15 g/L) ($P < 0.05$) (Supplemental Table 2). The number of children with inflammation (CRP >10 mg/L) was similar among children who received placebos (32) and supplements (28) (Supplemental

Table 2). The proportion of children admitted with diarrhea exhibiting inflammation was 43% less than the proportion of those admitted with pneumonia with inflammation (73%) ($P < 0.05$).

The duration of hospitalization was shorter in children who were receiving supplements than in those who were receiving placebos ($P < 0.05$) (**Table 2**). When the data of the children who died were included (intention to treat analysis), the duration of hospitalization was also shorter among children who received the supplement (7.0 ± 3.8 d) than in those taking the placebo (8.6 ± 4.8 d) ($P < 0.05$). This reduction in the period of hospitalization was independent of the children's ages. There was no significant interaction between admission diagnosis and treatment group with respect to duration of hospitalization. The duration of hospitalization among children admitted with diarrhea was 1.6 d (19%) shorter in the supplement group. Similarly, among children admitted with pneumonia, the duration of hospital stay was 1.9 d (20%) shorter in the group of children on supplements.

Table 2. Duration of hospitalization in HIV$^-$ infected children with diarrhea or pneumonia administered a supplement or placebo who completed the study [1]

	Diarrhea		Pneumonia		All children who completed	
	Placebo	Supplement	Placebo	Supplement	Placebo	Supplement
n	24	22	28	32	52	54
Hospitalization duration, d	8.6 ± 5.0	7.0 ± 4.5	9.4 ± 4.9	7.5 ± 3.5	9.0 ± 4.9	7.3 ± 3.9#

[1] Values are the mean ± SD. #Different from placebo group, $P < 0.05$

DISCUSSION

Our main objective in this study was to assess the effect of a commercially available multi-micronutrient supplement on the duration of acute diarrhea and of pneumonia in HIV-infected children who were not yet receiving ART. Duration of hospital stay was used as a measurement of the duration of acute diarrhea and of pneumonia. Supplementation indeed

reduced the length of hospitalization significantly by ~20%, (1.5 d), independent of admission diagnosis; a reduction that might be considered as clinically meaningful.

The nutritional status of the study children as measured by anthropometry and micronutrient status was poor. Mean values for WHZ indicate that a large portion of the children should be classified as wasted and mean values for HAZ demonstrate that most of the children were stunted (Table 1). Growth failure is common among HIV-infected children. The WAZ were significantly lower in HIV-infected children admitted with diarrhea to a South African academic hospital than in the uninfected control group (8). Stunting is said to be the growth problem most frequently seen in HIV-infected children (45).

The micronutrient status of the study children was poor as assessed by the absolute values of serum micronutrient concentrations compared to the local laboratory reference values (46), but there were no significant differences between the micronutrient concentrations of the supplement and placebo groups. Possibly the duration of the supplementation (only during hospitalization) was too short, but we did not collect serum samples before commencing treatment. The absence of such baseline serum concentrations hampers a valid interpretation of the lack of difference in micronutrient concentrations between the 2 treatment groups just before discharge. Anemia and low serum concentrations of micronutrients have been shown to be common among HIV-infected children (11, 12).

The percentage of children with anemia was significantly higher among children admitted with pneumonia than in children admitted with diarrhea. This is consistent with our observation that the proportion of children with inflammation (CRP >10 mg/L) was significantly higher among children admitted with pneumonia. Systemic infectious diseases (with resultant inflammation) are known to cause acute hemolytic anemia (47) and elevated CRP levels have shown to be highly correlated with anemia (48).

The proportion of children who died during hospitalization was 8% in the group of children admitted with diarrhea, whereas among those admitted with pneumonia it was 12%. This mortality rate of ~10% is similar to that observed among HIV-infected children in the general wards of our hospital. The anthropometry data suggests that the children who died were more stunted and underweight than the children who completed the study.

Despite a careful randomization, the children who were hospitalized with pneumonia and received the placebo treatment were 3.5 mo younger than those who received the supplement.

However, ANOVA revealed that age was not signifi cantly associated with duration of hospitalization. Therefore, it is unlikely that the difference in ages between the treatment groups might have confounded the treatment effect on the duration of hospitalization.

Supplemental Table 1. Anthropometric data of all enrolled HIV-infected children with diarrhea or pneumonia administered a supplement or placebo [1]

	Placebo		Supplement		All enrolled children	
	Completed study	Died	Completed study	Died	Placebo	Supplement
n	52	7	54	5	59	59
Age (mo)	10.5 ± 4.8	10.8 ± 3.3	13.2 ± 6.1	10.1 ± 4.8	10.6 ± 4.6	12.7 ± 6.0*
Weight (kg)	6.2 ± 1.5	5.5 ± 1.3	6.8 ± 1.6	5.4 ± 1.5	6.1 ± 1.5	6.7 ± 1.6*
Height (cm)	66.1 ± 7.3	62.1 ± 7.2	68.8 ± 7.1	65.0 ± 6.9	65.7 ± 7.3	68.5 ± 7.1*
WAZ	-2.77 ± 1.26	-3.47 ± 1.78	-2.68 ± 1.32	-3.50 ± 0.98	-2.85 ± 1.33	-2.75 ± 1.31
HAZ	-2.32 ± 1.59	-2.99 ± 2.93	-2.30 ± 1.61	-2.56 ± 1.98	-2.40 ± 1.78	-2.32 ± 1.63
WHZ	-1.53 ± 1.30	-1.69 ± 1.68	-1.61 ± 1.35	-2.31 ± 0.47#	-1.55 ± 1.33	-1.67 ± 1.31

[1] Values are the mean ± SD. * Different from placebo group. # Different from children who completed study

Supplemental Table 2. Biochemical indices of HIV-infected children with diarrhea or pneumonia administered a supplement or placebo who completed the study [1]*

	Diarrhea		Pneumonia		All children who completed	
	Placebo	Supplement	Placebo	Supplement	Placebo	Supplement
Serum zinc (µmol/L)	7.6 ± 2.7 (23)	7.6 ± 2.5 (21)	7.7 ± 2.7 (28)	8.6 ± 3.3 (32)	7.7 ± 2.7 (51)	8.2 ± 3.0 (53)
Serum retinol (µmol/L)	0.59 ± 0.29 (22)	0.76 ± 0.43 (21)	0.78 ± 0.39 (25)	0.76 ± 0.36 (31)	0.69 ± 0.36 (47)	0.76 ± 0.39 (52)
Serum iron (µmol/L)	7.6 ± 6.6 (23)	6.8 ± 3.3 (21)	7.6 ± 5.6 (28)	7.9 ± 5.4 (32)	7.6 ± 6.0 (51)	7.5 ± 4.7 (53)
Log serum ferritin (log [µg/L])	1.75 ± 0.56 (23)	1.75 ± 0.48 (21)	1.95 ± 0.66 (28)	1.85 ± 0.48 (32)	1.86 ± 0.62 (51)	1.81 ± 0.48 (53)
Hemoglobin (g/L)	98 ± 15 (22)	102 ± 16 (16)	88 ± 18 (28)	90 ± 18 (31)	93 ± 17 (50)	94 ± 18 (47)
Plasma CRP> [10 mg/L (%)	50 % (11)	40% (6)	75%(21))	71% (22)	64%(22)	61%(28)
CD4 percent	21.4 – 14.8 (22)	19.7 ± 10.1 (17)	19.9 ± 12.3 (27)	20.1 ± 13.0 (22)	20.1 ±13.0 (22)	20.6 ± 11.7 (39)

[1] Values are the mean ± SD (n) * No difference between the two treatment groups

As mentioned before, the duration of hospitalization was ~20% shorter in the treatment group and this was independent of the admission diagnosis. Unfortunately, there are hardly any studies that assess the effect of micronutrients in hospitalized HIV-infected children. Therefore, comparisons can only made with studies in hospitalized children from developing countries, where the nutritional status of the children might be similar to that found in HIV-infected children. Zinc supplements (combined with vitamin B complex) significantly reduced the stool output and duration of diarrhea in hospitalized Indian children compared with children given B complex vitamins only (49). Supplementation with a combination of zinc and copper to Indian children admitted with diarrhea did not significantly reduce the duration of hospitalization (50). Evidence suggests that zinc supplements have a beneficial effect on the duration of diarrhea, but the evidence for other micronutrients is equivocal. Analysis of pooled data indicated that zinc supplementation significantly reduces the duration of acute diarrhea (20). It is possible that the effects of the multi-micronutrient supplement (on the duration of diarrhea) that were observed in the current study are partly related to the zinc component. Nonetheless, the use of a multi-micronutrient supplement is likely to improve the overall micronutrient status of the children and may thus have greater benefits.

Among children admitted with pneumonia in the current study, those who received the supplement had a 20% shorter duration of hospitalization. In a group of children from India who were hospitalized with severe acute lower respiratory infection (ALRI), supplementation with zinc reduced the duration of symptoms of severity, but this effect was only noted in boys; vitamin A on the other had no effect on the symptoms (23). Some studies have shown no effect on the duration of hospitalization among children who were given zinc and/or vitamin A supplements (51), or only zinc supplements (24). A metaanalysis revealed that there is no evidence that high-dose vitamin A improves recovery from pneumonia in developing countries (29). Studies on the efficacy of zinc supplements in reducing the duration of pneumonia has shown conflicting results and as far as we know, no metaanalysis has been conducted on the effect of zinc on acute pneumonia. The use of the antioxidant vitamins E and C as adjunct therapy in children with severe ALRI has also been shown to have no benefit (52). While the benefits of a single micronutrient may be equivocal, a multi-micronutrient supplement as used in the current study may be more beneficial in HIV-infected children.

We consider the reduction in duration of hospitalization of 1.5 d as clinically meaningful. A longer duration of hospitalization has an impact on hospital bed occupancy and the risks of acquiring a nosocomial infection are also increased with longer hospitalization. Apart from the clinical importance there is also the health care relevance. In an academic hospital in South Africa, the total daily cost to the hospital of caring for an inpatient HIV-infected child in 2005 was estimated at $1007 (calculated by using mid-2005 South African Rand to US dollars exchange rates) (53), and the costs of the multi-micronutrient supplement are around $0.10 per day. We surmise that a reduction of 1.5 d in the duration of hospitalization wou ld result in significant cost savings per patient.

The major finding of our study is that short-term multi-micronutrient supplementation will significantly reduce the duration of hospitalization in HIV-infected children admitted with diarrhea or with pneumonia who are not yet treated with ART and who do not die during hospitalization. Whether supplementation with the multi-micronutrient for a longer period may also reduce the number of episodes of diarrhea and of pneumonia in HIV-infected children should be investigated.

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Chapter 4

Impact of multi-micronutrient supplementation on growth and morbidity of HIV-infected South African children

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Chapter 4

ABSTRACT

Background: Poor growth, micronutrient deficiencies and episodes of diarrhea and respiratory infections occur frequently in HIV-infected children. Deficiencies of micronutrients are associated with poor growth and increased risk of diarrhea and respiratory infections. The diarrhea and respiratory infections and poor growth in HIV-infected children may be partly related to the micronutrient deficiencies. Our objectives were to determine whether multi-micronutrient supplementation would improve the growth performance and reduce the number of episodes of diarrhea and/or of respiratory symptoms in HIV-infected children.

Methods: In a double-blind randomized trial, HIV-infected South African children aged 4-24 months (n = 201) were assigned to receive a multi-micronutrient supplement or placebo daily for six months. The children were assessed for respiratory symptoms or diarrhea bi-weekly; weights and heights were measured monthly.

Results: In total, 121 children completed the study (40% dropout). Dropouts were excluded from further analysis. Weight-for-height Z-scores improved significantly ($p<0.05$) among children given supplements compared to those given placebo (0.40 ± 1.22 versus -0.04 ± 1.40) (mean ± SD). Height-for-age Z-scores did not improve in both treatment groups. The number of monthly episodes of diarrhea in the placebo group (0.36 ± 0.36) was higher ($p=0.09$) than in the supplement group (0.25 ± 0.31) and the number of monthly episodes of respiratory symptoms was significantly higher ($p<0.05$) among children on placebos (1.01 ± 0.67) than those on supplements (0.66 ± 0.51).

Conclusion: Multi-micronutrient supplements significantly improved wasting but not stunting. Supplementation reduced the number of episodes of diarrhea and respiratory symptoms by about 30%.

INTRODUCTION

In 2008 there were 430 000 children younger than 15 years worldwide who were newly infected with HIV and 90% of whom were in Sub-Saharan Africa (1). Children who are infected with HIV are known to be at high risk for poor nutritional status, infections and poor growth performance (2, 3). Growth failure in HIV-infected children is associated with an increased risk of mortality (4, 5). Episodes of diarrhea and of respiratory symptoms appear more common in HIV-infected than in uninfected children (6).

Deficiencies of micronutrients, especially of vitamin A and zinc are associated with diarrhea and respiratory diseases (7, 8). Micronutrient status also influences growth. Deficiencies of zinc and of iron have been associated with impaired growth (9, 10). Vitamin A and carotenoid status were demonstrated to be independent predictors of growth failure in Malawian children (11). Micronutrient deficiencies seem to be common with HIV infection, and these deficiencies tend to be more severe in HIV-infected children (12, 13).

Studies on the effect of micronutrient supplementation on growth and morbidity in malnourished children have not always resulted in undeniably positive outcomes. Vitamin A supplementation has been shown to be beneficial in reducing the risk of diarrhea, but may be detrimental against respiratory infections (14). Zinc supplementation may improve growth (9) and reduce the incidence of diarrhea and respiratory infections in young children (15). Other studies indicate that zinc supplementation increases respiratory infections (16), although adding vitamin A seems to reduce the adverse effects of zinc on respiratory infections (17). Micronutrient deficiencies are seldom limited to a single micronutrient, therefore it has been suggested that supplements that contain an array of micronutrients should be provided (18).

Since HIV-infected children might be severely malnourished, they might profit most from beneficial effects of multi-micronutrient administration. Unfortunately, such studies are scarce. The aim of the present study was to investigate whether daily multi-micronutrient supplementation might improve the growth performance and might reduce the number of episodes of diarrhea or pneumonia in HIV-infected young South African children who were not yet on antiretroviral therapy (ART).

METHODS

The study was conducted from the Doctor George Mukhari Hospital in GaRankuwa, near Pretoria, the capital city of the Republic of South Africa.

HIV-infected children aged 4 to 24 months were enrolled into the study from the pediatric outpatient department of the hospital from November 2005 to November 2006.

Children who were on ART, or had received micronutrient supplementation or children with a chronic illness were not eligible for enrolment.

The Medunsa Research Ethics and Publications Committee approved the study; the permission of the hospital authorities was obtained, and the parents or guardians provided signed informed consent. The children were referred to the ART clinic when necessary as per standard hospital procedure (immunological and clinical staging was used) and taken off the study when ART was commenced.

In a double-blind, placebo controlled study, children were assigned to one of two intervention groups by computer randomization. The manufacturer prepared packs of tablets corresponding to the subject's number according to the randomization schedule. The investigators, field workers and participants were blinded to the treatment assignments, which were revealed after completing data analysis. The children had monthly appointments at the hospital for follow up weight and height measurements, collection of tablets, and evaluation of side-effects of micronutrients.

At enrolment and at the third and sixth monthly hospital visits, blood samples were taken for serum zinc, retinol, iron and ferritin and CD4 T-lymphocyte counts.

The tablets were prepared by the pharmaceutical company Adminicle Trading (Edenvale, South Africa). The multi-micronutrient supplement was a crushable tablet which contained 300 μg retinol, 0.6 mg thiamin, 0.6 mg riboflavin, 8 mg niacin, 0.6 mg pyridoxine, 1 μg cobalamin, 70 μg folic acid, 25 mg ascorbic acid, 5 μg 1,25-dihydrocholecalciferol, 7 mg d,l-tocopherol, 700 μg copper, 8 mg iron, 30 μg selenium, and 8 mg zinc at amounts based on recommended dietary allowances for a 1 year old child (19). The placebo and supplement tablets were identical in appearance and taste. The caregiver crushed the tablet using a pill crusher and mixed it with a small portion of the breakfast cereal which was fed to the child before the rest of the meal.

HIV tests: In children older than 15 months HIV-1 and HIV-2 serostatus was ascertained with ELISA tests (20); in younger children a PCR was performed additionally. The CD4 T-

lymphocyte counts were measured by means of a Coulter flow cytometer (Coulter Epics XL-MCL, Beckman Coulter) to stage the HIV infection.

Anthropometry: The subject's age was calculated from the date of birth. The weight was measured with the child wearing only light clothing to the nearest 0.1 kg, using one digital scale, calibrated using a standard weight of 10 kg. The length was measured on a baby board in the recumbent position to 0.1 cm. Z scores for weight-for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ) were calculated based on the National Centre for Health Statistics reference values using the Epi-Info software version 3.2.2 (21).

Blood sampling and analysis: Blood samples (8 ml) were collected after an overnight fast by venipuncture (site cleaned with trace element-free alcohol) from the cubital fossa or external jugular vein. Samples were collected in eight separate trace element-free tubes, with a removable non-rubber lid protected from light, sent to the laboratory immediately after collection, and stored at -20 C after centrifugation, until analysis. They were evaluated for hemolysis.

Serum zinc samples were measured by atomic absorption spectrometry (Perkin Elmer ICP/5500), serum retinol measurements by the fluorometric method (22), serum iron levels were measured by using rate spectrophotometry (SYNCHRON CX Systems IRON/TIBC Calibrator Kit, Beckman Instruments) and serum ferritin levels using commercial enzyme-linked immunoassay kits (Access Ferritin assay, Access Immunoassay Systems, Beckman Coulter). Quality control was performed by repeat analysis of standard reference material for low, normal and high values. The intra-assay and inter-assay coefficients of variation for serum zinc, retinol, iron and ferritin were all less than 5%.

Morbidity: The children were visited at home twice a week by a field worker who assessed whether the child was suffering from diarrhea or symptoms of acute respiratory tract infection, and when the symptoms resolved. Compliance was evaluated by pill count. The children were referred to hospital when necessary. Diarrhea was present on any day when the child passed three or more loose stools per day (23). Acute respiratory symptoms were defined as the history of cough and fever.

Statistical analysis:

Data entry and analyses were performed on SPSS 13.0 for Windows. Statistical analyses were two-tailed where appropriate and statistical significance was set at 5%. Data were assessed for normality and normalized by log transformation for serum ferritin.

The differences in anthropometric indices, and male to female ratios between the children who completed the study and those who dropped out were assessed by analysis of variance (ANOVA) and chi -square respectively.

Anthropometric indices, micronutrient concentrations and immunological parameters were studied by means of ANOVA for repeated mea sures with treatment group and gender as between subjects' factors. Changes in these measurements over 3 months and 6 months and differences between the two treatment groups in the number of diarrheal and pneumonia episodes per month were assessed using ANOVA by treatment group, age and gender.

RESULTS

A total of 201 children aged 4 to 24 months were enrolled (**Figure 1**); 121 completed the study. Among the 80 children who dropped out (40%), 43 died, 11 received treatment with ART, 13 relocated to another area and 13 dropped out for other reasons. Among the children who died, 80% died within the first month of follow up. The number of children who died and those who relocated was higher in the supplement group, while the number who received ART was higher in the placebo group, but none of these differences reached statistical significance.

The anthropometric indices and CD4 T-lymphocytes (at enrolment) of the study children who dropped out and those who completed the study are compared in **Table 1;** there were no differences in the ages and the ratio of males to females. The children who dropped out were significantly more stunted and underweight and marginally more wasted than those who completed the study, in both treatment groups. The percentages of CD4 T-lymphocytes of the children who dropped out were lower than those who completed the study; this was only significant in the placebo group.

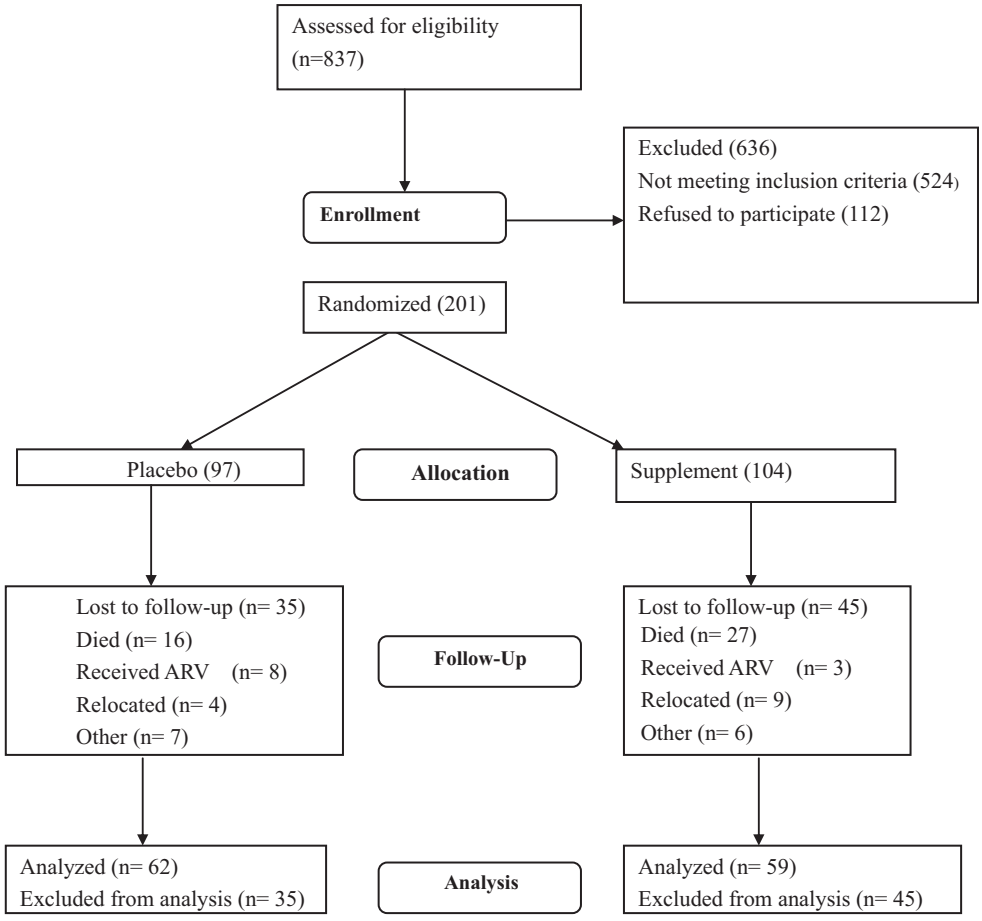

Figure 1. Profile of study participants

Table 1: Anthropometric indices and CD4 T-lymphocytes of children at enrolment

	Placebo		**Supplement**		**All enrolled children**	
	Children who completed	Children who dropped out	Children who completed	Children who dropped out	Children who completed	Children who dropped out
n	62	35	59	45	121	80
Male/Female	28/34	15/20	32/27	24/21	60/61	39/41
Age (months)	12.3 ± 6.5	11.7 ± 6.1	13.9 ± 6.1	12.8 ± 7.0	13.1 ± 6.4	12.3 ± 6.6
Weight (kg)	7.49 ± 1.86	6.37 ± 1.6#	7.89 ± 1.91	6.29 ± 1.7#	7.7 ± 1.9	6.3 ± 1.7#
Height (cm)	70.31 ± 7.59	66.06 ± 7.8#	72.92 ± 6.86$	67.59 ± 7.1#	71.6 ± 7.3	67.0 ± 7.4#
WAZ	-1.67 ± 1.61	-2.91 ± 1.27#	-1.98 ± 1.51	-3.00 ± 1.18#	-1.82 ± 1.56	-2.97 ± 1.21#
HAZ	-1.30 ± 1.66	-2.76 ± 1.68#	-1.23 ± 1.56	-2.43 ± 1.54#	-1.27 ± 1.61	-2.54 ± 1.57#
WHZ	-1.13 ± 1.24	-1.37 ± 1.36	-1.48 ± 1.27	-2.00 ± 1.32*	-1.30 ± 1.26	-1.46 ± 1.31#
CD4 percent (n)	28.0 ± 11.9 (48)	14.0 ± 8.9 (15)#	25.0 ± 12.5 (41)	21.1 ± 14.9 (16)	26.6 ± 12.2 (89)	17.7 ± 12.6 (31)#

Values presented as mean ± SD

$ Marginally different from subjects who completed study in placebo group (p=0.051)

#Significantly different from subjects who completed study (p<0.05)

*Marginally different from subjects who completed study (p=0.06)

Table 2: Immunological status of children over intervention period#

	Placebo		*Supplement*	
	n	***CD4 percentage***	***n***	***CD4 percentage***
Enrolment	42	29.6 ± 11.8	35	25.7 ± 13.1
3 mo	42	29.3 ± 12.7	35	24.1 ± 13.4
6 mo	42	28.0 ± 12.5	35	25.3 ± 13.8
Δ3mo	42	-0.51 ± 6.6	35	-1.23 ± 6.4
Δ 6 mo	42	-1.44 ± 7.5	35	-0.64 ± 8.3

Values presented as mean ± SD. #There were no significant differences between the 2 treatment groups in both absolute values and changes over 3 and 6 months

Table 3: Micronutrient status of the children over intervention period

	Placebo				***Supplement***			
	Zinc (µmol/L)	***Retinol (µmol/L)***	***Iron (µmol/L)***	***Log ferritin (log[µg/L])***	***Zinc (µmol/L)***	***Retinol (µmol/L)***	***Iron (µmol/L)***	***Log ferritin (log[µg/L])***
n	53	34	52	52	51	32	49	49
Enrolment	9.9 ± 2.9	0.77 ± 0.44	7.5 ± 4.0	1.60 ± 0.50	9.2 ± 2.8	0.85 ± 0.43	8.8 ± 4.5	1.52 ± 0.36
3 mo	10.0 ± 3.7	0.72 ± 0.23	9.1 ± 4.7	1.52 ± 0.45	10.6 ± 2.8	0.82 ± 0.37	11.3 ± 8.1	1.65 ± 0.33
6 mo	10.4 ± 3.4	0.72 ± 0.20	8.2 ± 5.7	1.54 ± 0.53	10.5 ± 3.3	0.85 ± 0.43	9.9 ± 5.6	1.61 ± 0.37
Δ3mo	0.07 ± 4.1	-0.05 ± 0.40	1.55 ± 4.8$	-0.08 ± 0.39$	1.37 ± 3.5$*	-0.03 ± 0.46	2.46 ± 9.22^	0.13 ± 0.4$#
Δ 6 mo	0.41 ± 4.0	-0.05 ± 0.48	0.67 ± 6.1	-0.06 ± 0.39	1.26 ± 4.1$	-0.01 ± 0.67	1.08 ± 7.23	0.10 ± 0.4*

Values presented as mean ± SD

$Significantly different from zero

^Marginally different from zero (p=0.07)

#Significantly different from placebo group (p<0.05)

* Marginally different from placebo group (p=0.09)

The immunological status of the children who completed the 6 months follow-up was poor as denoted by the CD4 T-lymphocyte percentage (**Table 2**). At enrolment about 50% of the children in the placebo and supplement groups had CD4 T-lymphocyte proportions below 25%. The CD4 T-lymphocyte percentage did not improve within both treatment groups and there was no significant difference between the two groups.

The concentrations of serum zinc, retinol, iron and ferritin over the 6 month period are presented in **Table 3**. There were no significant differences in the zinc and retinol concentrations between the two treatment groups at enrolment. The zinc concentrations improved significantly (by 14%) through the study period within the supplement group, but not significantly within the placebo group. Nonetheless, there was no significant difference in the absolute change of serum zinc concentrations between the two treatment groups. The retinol concentrations did not differ significantly through the 6 month intervention period within each treatment group and between the two treatment groups. The serum iron concentrations increased by 12.5% and the log ferritin by 0.1 in the supplement group. On the other hand, among children on placebos, the serum iron concentrations increased by 9% while the log ferritin value deteriorated significantly over the 6 months.

Weights and heights of the children who completed the follow-up period are shown in **Table 4** and their derived anthropometric indices in **Table 5**. The weights of the children increased significantly by 1.35 kg in the placebo group and by 1.66 kg in supplement group. Through the period of follow-up, there was a significant increase in height of 5.95 cm in the placebo group and 5.98 cm in the supplement group. Neither weight gain nor height increase was different between the two groups. There were no significant differences at enrolment between the two treatment groups in WAZ, HAZ and WHZ (**Table 5**), but the absolute low values of the Z-scores indicate that a large proportion of the children should be classified as wasted and stunted. WHZ as indicator for wasting and HAZ as indicator for stunting are graphically presented in **Figure 2**. Over the 6 month intervention there was a significant improvement in WAZ and WHZ values among the children in the supplement group but not in the placebo group. The change in HAZ did not differ between the groups.

The episodes of respiratory symptoms and of diarrhea were analyzed for children who had been followed up for 3 or more months. The number of episodes was then divided by the number of months of follow-up; these are presented in **Table 6**. The ages of these children

were similar in the two treatment groups. The children who received the supplements had fewer episodes of diarrhea per month (0.25 ± 0.31) (mean ± SD) compared to those who were given placebos (0.36 ± 0.36) ($p = 0.09$) and substantially lower number of episodes of respiratory symptoms: 0.66 ± 0.51 versus 1.01 ± 0.67 ($p<0.05$); the latter difference was independent of the children's ages.

DISCUSSION

The objective of the study was to assess the impact of 6 month multi-micronutrient supplementation on growth performance and number of episodes of diarrhea and respiratory symptoms of HIV-infected children.

There was a high dropout rate among the children in the study (40%), and among the children who dropped out the mortality was high at 54% (21% of all enrolled children). Although the number of children who died was higher in the group that received the supplements, 80% of children died within the first month of follow up in both treatment groups. It is therefore unlikely that the supplement was associated with the mortality. A pooled analysis of studies conducted in West, East and South Africa estimated that by two years of age 53% of HIV-infected children would have died (24). A survey that reviewed the mortality in a number of South African hospitals noted that approximately 48% of the deaths of children admitted to these hospitals were related to HIV infection (25). Only 11 (14% of the total number of dropouts) children were commenced on ART in the current study. The ART program was only rolled out in South Africa in 2004. The relatively low proportion of children who received ART in the study may be related to the fact that the rollout of the ART program was still at an early stage.

Children who dropped out were significantly more stunted and more wasted at enrolment than those who completed the study in both groups but the difference in wasting approached significance only among children who received the supplement. This chance finding occurred in spite of careful randomization procedures. Poor growth has been linked with increased mortality in HIV-infected children. This is supported by a study which indicated that wasting and stunting in HIV-infected children are associated with increased mortality (5). The immunological status (as measured by the percentage of CD4 T-lymphocytes) of the children

who dropped out in the current study was significantly worse than that of those who completed the study. This finding is in line with a meta-analysis of 10 studies that suggested that CD4 measurements are the most important indicator of mortality in HIV-infected children not receiving ART (26).

Supplementation with micronutrients over a period of six months did not have an effect on the immunological status as measured by the CD4 T-lymphocytes in the current study. This is in keeping with a study in which zinc supplementation in HIV-infected young South African children had no effect on the percentage of CD4 lymphocytes when compared to a placebo (27). In pregnant HIV-infected Tanzanian women, however, multivitamin supplementation significantly improved the women's CD4 counts, compared to women who received a placebo (28).

The concentrations of serum zinc improved significantly over the study period among children who received the multi-micronutrient supplement. However, the absolute change in serum zinc and retinol concentrations was not significantly different between the two treatment groups. On the other hand there was a significant difference in the change in log ferritin (it was higher in the supplement group). Unfortunately blood samples for acute phase reactants (e.g. C-reactive protein) were not taken; this might have hampered the assessment of the difference in micronutrient concentrations.

In the current study, the improvement in WAZ and WHZ over the six months period was significantly greater among children who were given the supplements. However the supplements had no effect on stunting. In Peru (an area with low HIV prevalence) no improvement was noted in WAZ and HAZ in children (age 6-35 months) who were given multi-micronutrients (29). Supplementation with vitamin A in HIV-infected Tanzanian children (6-60 months of age) significantly improved height, but not in uninfected children (30). It has been suggested that micronutrient supplements are more effective in children who have poor anthropometric indices. The children in the current study were more wasted and stunted than the children in the above noted studies, and all the supplements were given for six months except in the Tanzanian study where the duration was 12 months. A meta -analysis that assessed the effects of zinc supplementation on growth concluded that the effect was greatest in children older than six months whose initial HAZ were < -2.0 (9).

In the current study, children who received the micronutrient supplement had significantly fewer episodes of respiratory symptoms and fewer episodes of diarrhea than those who were on the placebo; the difference in diarrheal episodes did not reach statistical significance. In young (6-24 months of age) South African children who were born to HIV-infected mothers, receipt of a multi-micronutrient supplement (that included vitamin A) did not reduce the prevalence of diarrhea or respiratory symptoms when compared with children who were given vitamin A only irrespective of HIV status (2). However, multi-micronutrients supplementation reduced the incidence of diarrhea in rural South African children compared with those who received vitamin A only; the reduction was only noted in stunted HIV-uninfected children (31). Similarly, zinc supplements significantly reduced episodes of diarrhea in HIV-infected South African children; episodes of pneumonia however, were marginally reduced (27). A meta-analysis of 17 studies (from areas with low HIV prevalence) concluded that zinc supplementation significantly reduced the risk of diarrhea and respiratory illnesses (32).

Table 4: Weights and heights of the children over intervention period

	Placebo				Supplement			
	n	***Age (months)***	***Weight (kg)***	***Height (cm)***	***n***	***Age (months)***	***Weight (kg)***	***Height (cm)***
Enrolment	62	12.3 ± 6.5	7.5 ± 1.9	70.3 ± 7.6	59	14.0 ± 6.1	7.9 ± 1.9	72.9 ± 6.9~
1 mo	62	13.3 ± 6.5	7.7 ± 1.9	71.1 ± 7.4	59	15.2 ± 6.4	8.3 ± 1.8	73.9 ± 6.9#
2 mo	62	14.2 ± 6.5	8.0 ± 1.9	72.1 ± 7.2	59	16.1 ± 6.4	8.5 ± 1.9	74.8 ± 6.9#
3 mo	62	15.1 ± 6.5	8.3 ± 1.9	73.1 ± 7.1	59	17.1 ± 6.4	8.8 ± 2.0	75.8 ± 7.0#
4 mo	62	16.1 ± 6.5	8.5 ± 2.0	74.2 ± 7.1	59	18.0 ± 6.4	9.0 ± 2.0	76.9 ± 7.0#
5 mo	62	17.0 ± 6.6	8.6 ± 2.1	75.1 ± 7.1	59	19.0 ± 6.4	9.3 ± 1.9*	77.8 ± 7.0#
6 mo	62	17.9 ± 6.6	8.8 ± 2.1	76.3 ± 7.2	59	19.9 ± 6.4	9.6 ± 2.0^	78.9 ± 7.1#
Δ3 mo	62	2.9 ± 0.4$	0.80 ± 0.60$	2.83 ± 1.54$	59	3.0 ± 0.4$	0.89± 0.75$	2.89 ± 1.59$
Δ 6 mo	62	5.6 ± 0.5$	1.35 ± 0.96$	5.95 ± 2.24$	59	5.8 ± 0.5$	1.66 ± 1.10$	5.98 ± 2.31$

Values presented as mean ± SD

$Significantly different from zero ($p<0.001$)

#Significantly different from placebo group ($p<0.05$)

~Marginally different from placebo group ($p=0.051$)

*Marginally different from placebo group ($p=0.09$)

^Marginally different from placebo group ($p=0.06$)

Table 5: Anthropometric indices of the children over intervention period

	Placebo					Supplement				
Follow up phase	***n***	***Age (months)***	***WAZ***	***HAZ***	***WHZ***	***n***	***Age (months)***	***WAZ***	***HAZ***	***WHZ***
Enrolment	62	12.3 ± 6.5	-1.67 ± 1.61	-1.30 ± 1.66	-1.12 ± 1.24	59	14.0 ± 6.1	-1.98 ± 1.51	-1.23 ± 1.56	-1.48 ± 1.27
1 mo	62	13.3± 6.5	-1.75 ± 1.57	-1.40 ± 1.70	-1.10 ± 1.13	59	15.2 ± 6.4	-1.86 ± 1.41	-1.34 ± 1.46	-1.30 ± 1.15
2 mo	62	14.2 ± 6.5	-1.79 ± 1.60	-1.48 ± 1.69	-1.07 ± 1.19	59	16.1 ± 6.4	-1.83 ± 1.44	-1.41 ± 1.47	-1.22 ± 1.18
3 mo	62	15.2 ± 6.5	-1.76 ± 1.58	-1.50 ± 1.64	-1.00 ± 1.17	59	17.1 ± 6.4	-1.79 ± 1.49	-1.38 ± 1.46	-1.18 ± 1.25
4 mo	62	16.1 ± 6.5	-1.80 ± 1.59	-1.46 ± 1.62	-1.10 ± 1.23	59	18.0 ± 6.4	-1.77 ± 1.45	-1.31 ± 1.46	-1.23 ± 1.15
5 mo	62	17.0 ± 6.6	-1.84 ± 1.64	-1.45 ± 1.71	-1.17 ± 1.25	62	19.0 ± 6.4	-1.70 ± 1.42	-1.28 ± 1.53	-1.15 ± 1.11
6 mo	62	18.5 ± 7.1	-1.85 ± 1.69	-1.26 ± 1.66	-1.27 ± 1.18	59	20.0 ± 6.5	-1.58 ± 1.52	-1.14 ± 1.59	-1.08 ± 1.24
Δ3 mo	62	2.9 ± 0.4$	-0.11 ± 0.69	-0.20 ± 0.55$	0.08 ± 0.92	59	3.0 ± 0.4$	0.11 ± 0.80^#	-0.18 ± 0.84	0.21 ± 0.91$
Δ 6 mo	62	5.6 ± 0.5$	-0.19 ± 1.10	0.03 ± 0.88	-0.04 ± 1.40	59	5.8 ± 0.5$	0.39 ± 1.07$#	0.09 ± 0.95	0.40 ± 1.22$#

Values presented as mean ± SD

$Significantly different from zero ($p<0.05$)

^Marginally different from zero ($p=0.07$)

#Significantly different from placebo group ($p<0.05$)

Figure 2: Anthropometric Z-scores of children who completed study

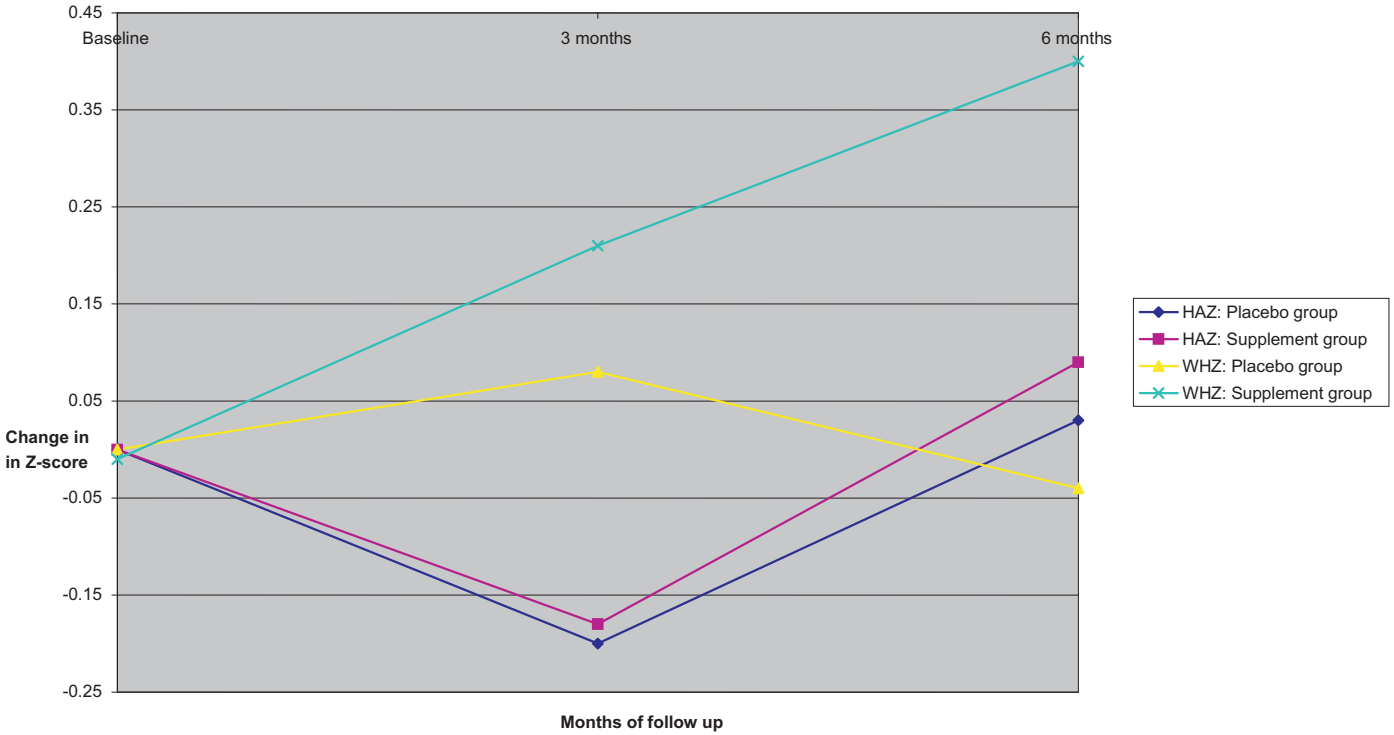

Table 6: Diarrhea and respiratory symptom episodes per month of follow up

	n	*Age at enrolment (months)*	*Number of diarrheal episodes per month*	*Number of episodes of respiratory symptoms per month*
Placebo	53	12.0 ± 6.3	0.36 ± 0.36	1.01 ± 0.67
Supplement	52	13.7 ± 6.0	0.25 ± 0.31*	0.66 ± 0.51#

Values presented as mean ± SD

*Marginally different from placebo group (p=0.087)

#Significantly different from placebo group (p<0.05)

The improvement in growth performance and reduction in morbidity that was observed in this study suggests that micronutrient supplements are useful as adjunct therapy in HIV-infected children. While there was no effect on CD4 lymphocytes, there were no deleterious effects. At the time of conducting the study, ART was not widely available at the local hospital, but has since become available to an increasing number of children in sub-Saharan African countries. However, because ART are given to children with advanced HIV disease, micronutrient supplementation may be useful in those children who are as yet not eligible for ART. The effect of micronutrient supplementation in children in children who are on ART should also be investigated.

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Chapter 5

Improved appetite after multi-micronutrient supplementation for six months in HIV-infected South African children

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Chapter 5

Abstract

The aim of the study was to assess the effect of multi-micronutrient supplementation on the appetite of HIV-infected children. HIV-infected children (6-24 months) who had previously been hospitalized were enrolled into a double-blind randomized trial, and given daily multi-micronutrient supplements or placebos for six months. Appetite tests were performed at enrolment and after three and six months. Appetite was measured as *ad libitum* intake of a commercial cereal test food served after an overnight fast according to standardized procedures. Body weights and total amount of test food eaten were measured. In total, 99 children completed the study (50 on supplements and 49 on placebos). Amounts eaten per kilogram body weight in the supplement group at enrolment and after six months were 36.7 ± 17.7 g/kg (mean ± SD) and 41.3 ± 15.0 g/kg respectively, while the amounts in the placebo group were 47.1 ± 14.9 g/kg and 45.7 ± 13.1 g/kg respectively. The change in amount eaten per kilogram body weight over six months was significantly higher in the supplement group (4.7 ± 14.7 g/kg) than in the placebo group (-1.4 ± 15.1 g/kg). Multi-micronutrient supplementation for six months seems to significantly improve the appetite of HIV-infected children.

INTRODUCTION

Severe malnutrition is a frequent finding in children who are infected with HIV (Kessler, Daley, Malenga & Graham, 2000). Undernutrition has indeed been found to be a major problem in HIV-infected children in South Africa as illustrated by a study that revealed that more than 65% of these children were stunted before starting antiretroviral (ARV) therapy (Eley et al., 2006).

The origin of malnutrition in HIV infection is considered multi-factorial, and the mechanisms are thought to include decreased energy intake (Arpadi et al., 2000), nutrient malabsorption (Knox, Spiegelman, Skinner & Gorbach, 2000) and increased resting energy expenditure per kilogram of fat-free mass (Batterham, 2005). Reduced energy intake is considered to be a primary contributing factor to the pathogenesis of HIV-associated wasting (Mulligan et al., 2007). Asymptomatic HIV-infected children have been shown to have lower dietary energy and protein intakes compared to uninfected children who were born to HIV-infected mothers (Jahoor, Abramson & Heird, 2003). Decreased nutrient intake in HIV-infected children may result from anorexia (loss of appetite), central nervous system disease and dysphagia; these have been summarized in the review by Semba and Tang (1999). In a group of HIV-infected Indian children loss of appetite was a common symptom, with 59% of the children reported to have this symptom (Pol, Shepur & Ratageri, 2007). Loss of appetite was reported to be common among HIV-infected adults in Côte D□Ivoire (Castetbon et al., 1997). Oesophageal candidiasis is common with HIV infection and will often result in dysphagia (difficulty in swallowing) and odynophagia (painful swallowing) and these in turn may also contribute to reduced energy and nutrient intake (Semba & Tang, 1999). Malnutrition in HIV-infected children is therefore likely to be related at least partly to the poor appetite of these children.

Poor appetite might be caused by micronutrient deficiency (Lawless, Latham, Stephenson, Kinoti, & Pertet, 1994; Umeta, West, Haidar, Deurenberg, & Hautvast, 2000), and since micronutrient deficiencies are common among HIV-infected children (Eley, Sive, Abelse et al., 2002; Eley, Sive, Shuttleworth, & Hussey, 2002), it is possible that the poor appetite in these children is to a degree influenced by these deficiencies. Zinc deficiency has been associated with disturbances in taste perception and appetite (Chen, Song, & Lin, 2000;

Ueda et al., 2006). A strong association between anorexia nervosa and zinc deficiency has been established (Shay & Mangian, 2000). Zinc supplements given to stunted Ethiopian children significantly improved appetite compared to children who were given a placebo (Umeta et al., 2000). Iron deficiency is also known to be associated with poor appetite. It has been reported that iron supplementation improved appetite and growth in anaemic Kenyan children (Lawless et al., 1994).

Another cause of poor appetite might be inadequate hormonal regulation of appetite. Insulin and leptin, for example, are involved in appetite regulation. The hormone leptin is primarily produced in the adipose tissue and is positively associated with body fat mass and recent energy intake. Circulating leptin levels signal to the brain that body fat stores and energy intake are adequate and this will result in appetite suppression. Insulin stimulates leptin production and secretion (Arsenault et al., 2007). Whether the effect of these hormones on appetite is influenced by micronutrient levels is unclear. Some studies have suggested that zinc may be involved in the regulation of serum leptin concentrations (Chen et al., 2000; Mantzoros et al., 1998).

Since micronutrient deficiencies might result in lowered appetite of HIV-infected children possibly through appetite regulating hormones, we conducted a randomized placebo-controlled trial to assess the effect of multi-micronutrient supplementation on the appetite of these children and their levels of appetite regulating hormones.

SUBJECTS AND METHODS

Subjects and study area

The study was conducted from the Doctor George Mukhari hospital which is located about 30 km northwest of Pretoria, the capital city of the Republic of South Africa. This academic public hospital is attached to the Medunsa campus of the University of Limpopo and serves an area of approximately 2600 square km, including the township of Soshanguve where about one million people live.

HIV-infected children aged 6 to 24 months who had been previously admitted to the paediatric wards of the hospital with pneumonia or diarrhoea were enrolled into the study.

The children were recruited either on discharge from the wards or at the follow up visit (post discharge) to the paediatric outpatient department (POPD) of the hospital.

Children who were on anti-retroviral (ARV) therapy or had received micronutrient supplementation in the previous two months were not eligible for enrolment into the study. Similarly, children who had been diagnosed with a chronic illness unrelated to HIV infection were excluded. The children were enrolled from November 2005 until November 2006.

***Ethical considerations*:**

The Research Ethics and Publications Committee of the Faculty of Medicine at Medunsa approved the study, and the permission of the hospital authorities was obtained. The parents or guardians provided signed informed consent. The children were referred to the anti-retroviral (ARV) clinic when required in line with standard hospital procedures by using immunological and clinical staging. A child was only removed from the study when the physicians at the ARV clinic decided to commence ARV therapy (this was the case in eight of the children, see Fig. 1).

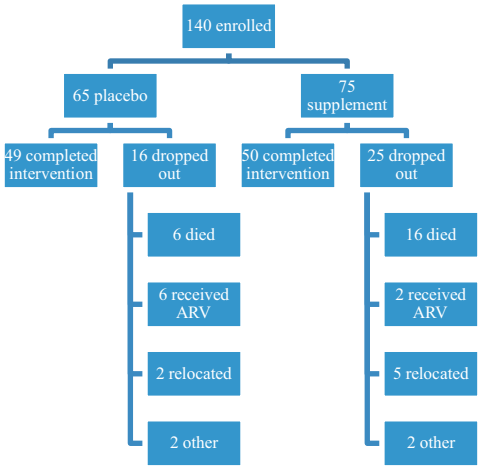

Figure 1: Profile of enrolled children

Study design

A double-blind, placebo controlled study was performed. Children were randomly assigned to one of two intervention groups using a simple randomization schedule by means of computer generated numbers. A staff member of the university attached to the hospital and who was not involved with the study did the randomization. The manufacturer prepared numbered packs of tablet corresponding to the subject's number according to the randomization schedule. The investigators, field workers and participants were all blinded to the treatment assignments.

The tablets were given to the children's caregivers to administer to the children dai ly for six months. The study children were also given appointments for monthly hospital follow up visits, during which weight and height measurements were performed.

At enrolment, and at the third and sixth monthly hospital visits, appetite tests were performed and blood samples were taken for serum zinc, iron, ferritin, as well as plasma insulin and leptin.

Intervention

The multi-micronutrient supplement and placebo were prepared by the pharmaceutical company Adminicle Trading (Edenvale, South Africa). The multi-micronutrient supplement contained vitamin A 300 µg, vitamin B1 0.6 mg, vitamin B2 0.6 mg, vitamin B3 8 mg, vitamin B6 0.6 mg, vitamin B12 1 µg, folic acid 70 µg, vitamin C 25 mg, vitamin D 5 µg, vitamin E 7 mg, copper 700 µg, iron 8 mg, selenium 30 µg and zinc 8mg. These values are based on the recommended daily allowances for a one year old child (Food and Nutrition Board-Institute of Medicine, 2000). The placebo and supplement tablets were identical in appearance and were administered by crushing (using a pill crusher) and dissolving in a small amount of water.

Methods

HIV tests

HIV-1 and HIV-2 serostatus was ascertained with the use of ELISA tests in children older than 15 months (Urassa, Matunda, Bredberg-Raden, Mhalu & Biberfeld, 1994). In children younger than 15 months a PCR test was performed in addition to the ELISA tests (van der Weert et al., 2006). The CD4 T-lymphocyte counts were measured by means of a Coulter

flow cytometer (Coulter Epics XL-MCL, Beckman Coulter) to assess immunological stage of HIV infection.

Table 1

Weights and heights of the children throughout the intervention

	Age (months)	Weight (kg)	Height (cm)
Placebo (n = 49)			
Enrolment	13.6 ± 5.7	7.9 ± 1.7	72.2 ± 6.3
3 mo	16.4 ± 5.7	8.7 ± 1.7	74.8 ± 6.1
6 mo	19.3 ± 5.7	9.3 ± 1.9	77.9 ± 5.9
Δ 3 mo	O.V – M.4A	M.UP – M.SMA	O.SO – 1.4PA
Δ 6 mo	R.T – M.SA	1.PR – M.VSA	R.VR – O.O4A
Supplement (n = 50)			
Enrolment	15.1 ± 5.4	8.1 ± 1.9	74.3 ± 6.0
3 mo	18.2 ± 5.7	9.0 ± 2.0	77.1 ± 6.5*
6 mo	21.1 ± 5.6	9.8 ± 2.1	80.0 ± 6.8
Δ 3 mo	3.1 ± 0.7$	0.89 ± 0.78$	2.78 ± 1.61$
Δ 6 mo	6.0 ± 0.7$	1.66 ± 1.10$	5.98 ± 2.31$

Values presented as mean ± SD. $Significantly different from zero ($P < 0.001$)

*Marginally different from placebo group ($P = 0.08$)

Anthropometry

The subject's age was calculated in months from the date of birth as given by the mother. The weight was measured without shoes, and with the child wearing only light clothing to the

nearest 0.1 kg using the same single digital scale for all children. The scale was placed on a table and was calibrated before each measurement session using a standard weight of 10 kg.

The length was measured in the recumbent position to 0.1cm, on a baby board, by the investigator (SM) with the help of an assistant. One examiner held the child's head (with the chin in the neutral position) in contact with the fixed part of the board while the other examiner stretched the child to maximum length and then brought the movable part of the board into contact with the heels.

Z scores for weight-for-age, height-for-age, and weight-for-height were calculated based on the National Centre for Health Statistics (NCHS) reference values by means of the Epi-Info software version 3.2.2 (Dean *et al*., 2000).

Blood sampling and analysis

All blood samples were collected after an overnight fast by venipuncture (puncture site cleaned with trace element free alcohol). Approximately 8 ml of blood was collected from each child. When the cubital fossa veins were not easily accessible, blood was obtained from the external jugular vein using a stainless steel needle and a plastic syringe. The samples were collected in eight separate trace element free tubes, with a removable non-rubber lid. The needle was removed from the syringe prior to injecting the blood into tubes in order to limit haemolysis. The samples were evaluated for haemolysis. All the blood samples were collected and sent to the laboratory immediately after collection, protected from light, and stored at -20°C after centrifugation, until analysis. The samples were analyzed within three months of collection at the Medunsa branch of the national Health Laboratory Service NHLS).

Serum zinc samples were measured by atomic absorption spectrometry in µmol/ (Perkin Elmer ICP/5500), separation from cell was conducted within 45 minutes. Quality control was assessed by repeat analysis of standard reference material. Coefficients of variation of less than 5% were considered acceptable. Serum iron concentrations were measured in µmol/l by using rate spectrophotometry (SYNCHRON CX Systems IRON/TIBC Calibrator Kit, Beckman Instruments). Quality control was assessed by repeat analysis of standard reference material for low, normal and high values. Serum ferritin was measured by using commercial ELISA kits in µg/l (Access Ferritin assay, Access Immunoassay Systems, Beckman Coulter). Similarly quality control was assessed by repeat analysis of standard reference material for

low, normal and high values. Coefficients of variation of less than 5% were considered acceptable. Plasma insulin concentrations were measured using enzyme labelled immunometric assays in mU/L, and plasma leptin concentrations were measured with the use of radioimmunoassay in ng/ml.

Table 2

Anthropometric indices of the children throughout the intervention

	Age (months)	WAZ	HAZ	WHZ
Placebo (n = 49)				
Enrolment	13.6 ± 5.7	-1.76 ± 1.63	-1.27 ± 1.65	-1.30 ± 1.28
3 mo	16.4 ± 5.7	-1.68 ± 1.52	-1.42 ± 1.62	-1.03 ± 1.21
6 mo	19.3 ± 5.7	-1.67 ± 1.65	-1.09 ± 1.53	-1.22 ± 1.27
Δ 3 mo	2.9 ± 0.4$	0.08 ± 0.61	-0.15 ± 0.53^	0.27 ± 0.86$
Δ 6 mo	5.7 ± 0.6$	0.10 ± 1.00	0.18 ± 0.80	0.08 ± 1.16
Supplement (n = 50)				
Enrolment	15.1 ± 5.4	-2.11 ± 1.50	-1.27 ± 1.56	-1.63 ± 1.19
3 mo	18.2 ± 5.7	-1.84 ± 1.50	-1.38 ± 1.50	-1.28 ± 1.23
6 mo	21.1 ± 5.6	-1.59 ± 1.52	-1.13 ± 1.63	-1.11 ± 1.22
Δ 3 mo	3.1 ± 0.7$	0.27 ± 0.78$	-0.11 ± 0.90	0.36 ± 0.90$
Δ 6 mo	6.0 ± 0.7$	0.52 ± 1.00$#	0.14 ± 0.95	0.53 ± 1.22$*

Values presented as mean ± SD. $Significantly different from zero ($P < 0.05$). ^Marginally different from zero ($P = 0.06$). #Significantly different from placebo group ($P < 0.05$). * Marginally different from placebo group ($P = 0.06$)

Appetite testing

Conditions prior to testing: The children were given appointments on two separate days within a period of two weeks. The child was not allowed to eat any food (not even breast or formula milk) from the last meal of the day preceding the day of the appetite test, until after the appetite test. The test was conducted early in the morning. Compliance with these conditions was ascertained and if for any reason the mother had fed the child prior to the appetite test, another date for appetite testing was set.

Test food: A honey and wheat cereal, Nestlé Nestum® No 2 was used; in each case 75 gm of dry porridge was weighed on an electronic load cell scale to the nearest 0.1 gm. The preparation of the test cereal was standardised. Fresh full fat milk in a 2 litre milk container was put in a bowl of boiled water, until the milk T reached 50 C, (measured by using a metal stem thermometer) 480 ml of this milk was then added to the dry porridge and mixed to a uniform consistency. Each 100 gm of the dry cereal contains 9.3 gm protein, 77.9 gm carbohydrates, 1.7 gm fat, and 5.2 gm fibre and have 1634 kJ of energy. One hundred millilitres of the milk contains 3.2 gm protein, 4.8 gm carbohydrates and 3.8 gm fat and yields 260 kJ.

Test feeding procedure: A porcelain bowl that easily accommodates a 700 ml volume was used. Plastic teaspoons were used to feed the children. The weight of the wet cereal was measured. An empty bowl was measured first and then the bowl with the cereal was measured. The test was conducted in a quiet room, with three to five mother and child pairs at a time. Each pair sat apart from the next, separated from the other participants by a curtain. The mothers were not allowed to communicate with each other during the test and they could not see how the other children were eating. The mother helped her child such that the child was eating *ad libitum;* the mother was not allowed to verbally encourage the child to eat, nor to apply any kind of pressure on the child. The mothers were informed that the child was not obliged to finish the cereal. When the child stopped eating, the amount eaten and the duration of the eating episode were noted. A five minute break was then given, after which the child was invited to continue eating. The bowl with the leftover cereal was then weighed. An observer was on hand to ensure strict adherence to the procedures. The observer also recorded the duration of each eating episode. The total amount eaten and the total duration of the eating episode were used for further analysis.

Statistical analysis

Data elaboration and analysis was performed by SPSS 13.0 for Windows. Statistical significance was set at 5%.

Data were assessed for normality by visual examination of distribution plots, followed by normality tests. Where appropriate data were normalized by log transformation and these log transformed values were used for analysis. Serum ferritin and plasma insulin concentrations were log transformed.

The differences in baseline anthropometric indices, and ratio of males to females between the children who completed the study and those who dropped out were compared by univariate analysis of variance (ANOVA) and the chi-square test respectively.

ANOVA revealed that there was no systematic difference between appetite measurements done on the first and second day of appetite testing within the two week period. Consequently mean values were calculated for each subject, and these mean values were utilized in the analysis. Changes in appetite measurements, anthropometric parameters, micronutrient concentrations and hormone levels were assessed by using ANOVA for repeated measures with treatment group and gender as between subjects factors. The changes in the parameters over three months and over six months were also tested by means of univariate ANOVA.

RESULTS

A total of 140 children aged 6-24 months were enrolled. Ninety-nine children completed the study (29% dropout). Among the 41 children who dropped out, 22 died, eight were started on anti-retroviral (ARV) therapy, seven relocated to another area, and the other four dropped out for other reasons (Fig. 1). Although the number of children who died and the number who relocated was higher in the group of children who received the supplement, these differences did not reach statistical significance. The number of the ones who were started on ARVs was higher in the placebo group and this too was not statistically significant. The children who dropped out and those who completed the study were not significantly different in terms of age (14.4 ± 6.4 months compared to 14.3 ± 5.5 months) (mean ± SD) and the ratio of males to females (0.77 as opposed to 0.86).

The weights and heights of the subjects who completed the six month follow up period are shown in Table 1 and their derived anthropometric indices in Table 2. The male to female ratio among the children who completed the six month intervention was not significantly different between the two treatment groups (0.81 and 0.72 in the placebo and supplement groups respectively). There were no significant differences at enrolment between the two treatment groups in terms of weight and height. The increase of the weights of the children of 1.4 kg in the placebo group and 1.7 kg in the supplement group was not significantly different between the two groups. Over the six-month period of follow up the height increased by 5.95 cm and 5.98 cm in the placebo and supplement groups respectively. There were no significant differences at enrolment between the two treatment groups in the weight-for-age Z-score (WAZ), height-for-age Z-score (HAZ) and weight-for-height Z-score (WHZ). However, a large proportion (46%) of the enrolled children were wasted and/or stunted. A total of 32 of these children were stunted (HAZ < -2SD) and 33 were wasted (WHZ < -2 SD), with 19 of them found to be both wasted and stunted. During the six-month intervention there was a significant improvement in the WAZ and WHZ among the children in the supplement group, and this improvement was significantly better than that in the placebo group. There were no significant changes in the HAZ over the six-month period in both treatment groups.

The serum concentrations of zinc, iron and ferritin over the six month period are presented in Table 3. There were no significant differences in these serum concentrations between the two groups at enrolment. Over the six month period the serum zinc concentrations improved significantly (by 12%) within the supplement group, but not within the placebo group. However, there was no significant difference in the absolute change of serum zinc concentrations over six months between the two treatment groups. The log ferritin values improved over the intervention period in the supplement group, and this improvement reached statistical significance within the first three months. On the other hand, the log ferritin value in the placebo group deteriorated significantly over the first three months, and did not change in the next three months.

The plasma leptin and the log transformed insulin concentrations over the study period are shown in Table 4. There were no significant changes in these values, either within each group or between the two groups.

Table 3

Micronutrient status of the children throughout the intervention

	Zinc (μmol/L)	Iron (μmol/L)	Log ferritin (log[μg/L])
Placebo			
n	43	43	43
Enrolment	9.7 ± 2.8	7.0 ± 4.1	1.49 ± 0.44
3 mo	9.4 ± 3.1	9.0 ± 4.8	1.41 ± 0.36
6 mo	10.1 ± 3.0	8.3 ± 5.8	1.41 ± 0.41
Δ 3 mo	-0.25 ± 3.84	2.08 ± 4.86$	-0.08 ± 0.42
Δ 6 mo	0.45 ± 3.80	1.36 ±5.99	-0.08 ± 0.51
Supplement			
n	44	42	42
Enrolment	9.4 ± 2.8	8.6 ± 4.3	1.49 ± 0.33
3 mo	10.6 ± 2.8	11.8 ± 8.4	1.64 ± 0.34
6 mo	10.5 ± 3.1	9.9 ± 5.6	1.61 ± 0.39
Δ 3 mo	1.19 ±3.46$*	3.23 ± 9.21$	0.15 ±0.37$#
Δ 6 mo	1.15 ± 3.76$	1.30 ± 7.44	0.11 – 0.40^#
NHLS reference values (NHLS, 2007)	8.2 – 23.0	9.0- 21.5	0.85 - 2.15

Values presented as mean ± SD. $ Significantly different from zero. ^Marginally different from zero ($P = 0.07$). #Significantly different from placebo group ($P < 0.05$)

* Marginally different from placebo group ($P = 0.07$)

The amounts of test food eaten (in grams and in grams/kg body weight), eating duration and eating rate are presented in Table 5. The amount of test food eaten at enrolment (in grams and in amount per kg body weight) was significantly ($p<0.05$) higher in the placebo group compared to that in the supplement group. The change in amount of test food eaten (either expressed in grams or in grams/kg body weight) over the six months follow up period was significantly higher in the supplement group ($p<0.05$).

DISCUSSION

The main objective of the study was to investigate whether a six month multi-micronutrient supplementation would improve the appetite of HIV-infected young South African children. We indeed observed a significant improvement in appetite as measured by the amount of test food eaten.

In the current study appetite was measured by *ad libitum* consumption of a commercial cereal test food after an overnight fast. This technique of appetite testing has been validated in young Beninese children as an appropriate tool for testing and evaluating appetite (Dossa, Ategbo, van Raaij, de Graaf, & Hautvast, 2002a). Even though the children in the current study were younger and more malnourished than the Beninese children, the standardization of the testing procedures was successfully implemented.

The improved appetite was observed in a group of children who were poorly nourished at enrolment, as signified by the anthropometric indices which showed that 46% of them were malnourished (stunted and/or wasted), and also indicated by the serum values of the micronutrients relative to the local laboratory reference values (NHLS, 2007).

The improvements in appetite were not associated with changes in the hormones leptin and insulin. It should, however, be realized that the leptin concentrations were tested in a small number of children and thus there was insufficient power to arrive at a definite conclusion. Nonetheless a study conducted in young Peruvian children indicated that zinc supplementation over six months had no effect on the leptin, ghrelin and insulin concentrations of the children (Arsenault et al., 2007).

Table 4

Hormone levels of the children throughout the intervention

	Log Insulin (log[mU/L])	Leptin (ng/ml)
Placebo		
n	42	14
Enrolment	0.10 ± 0.34	1.60 ± 1.06
3 mo	0.22 ± 0.47	2.55 ± 0.75
6 mo	0.11 ± 0.41	1.84 ± 0.66
Δ 3 mo	0.12 ± 0.64.	0.95 ± 0.96$
Δ 6 mo	0.01 ± 0.58	0.24 ± 1.36
Supplement		
n	41	17
Enrolment	0.06 ± 0.44	1.74 ± 1.43
3 mo	0.25 ± 0.54	2.00 ± 0.94*
6 mo	0.09 ± 0.51	1.81 ± 1.14
Δ 3 mo	0.19 ± 0.73	0.26 ± 1.24
Δ 6 mo	0.03 ± 0.64	0.06 ± 1.73

Values presented as mean ±SD. * Marginally different from placebo group ($P = 0.08$).

$Significantly different from zero

Table 5

Appetite of the children throughout the intervention

	Age (months)	Amount eaten (g)	Eating duration (min)	Eating rate (g/min)	Amount eaten (g/kg Bwt)
Placebo (n = 49)					
Enrolment	13.6 ± 5.7	362 ± 124	25.4 ± 7.3	15.3 ± 6.5	47.1 ± 14.9
3 mo	16.4 ± 5.7	405 ± 129	26.4 ± 8.1	16.5 ± 7.2	47.6 ± 15.5
6 mo	19.3 ± 5.7	419 ± 137	26.0 ± 9.2	17.8 ± 8.3	45.7 ± 13.1
Δ 3 mo	2.9 ± 0.4$	43 ± 98$	1.1± 6.4	1.2 ± 5.6	0.5 ± 13.1
Δ 6 mo	5.7 ± 0.6$	57 ± 130$	0.7 ± 9.1	2.5 ± 7.6$	-1.4 ± 15.1
Supplement (n = 50)					
Enrolment	15.1 ± 5.4	286 ± 130#	24.2 ± 9.0	12.4 ± 5.7#	36.7 ± 17.7#
3 mo	18.2 ± 5.7	364 ± 141	25.3 ± 7.5	15.5 ± 8.3	42.0 ± 17.4
6 mo	21.1 ± 5.6	394 ± 139	25.6 ± 8.8	16.9 ± 7.6	41.3 ± 15.0
Δ 3 mo	3.1 ± 0.7$	79 ± 120$^	1.1 ± 11.3	3.1 ± 7.8$	5.3 ± 14.9$
Δ 6 mo	6.0 ± 07.7$	108 ± 112 #	1.4 ± 11.6	4.5 ± 7.2$	4.7 ±14.7$

Values presented as mean ± SD. $ Significantly different from zero ($p<0.05$).

^ Marginally different from placebo group ($p=0.08$). * Marginally different from placebo group ($p=0.09$). # Significantly different from placebo group ($p<0.05$).

Children who received the supplements achieved significant improvements in their appetites over the six month period. This was in relation to amount of test food eaten, both in terms of absolute amounts, and amounts per kg body weight. A number of published studies have demonstrated that appetite stimulants (like megestrol acetate) can improve the appetite of HIV-infected subjects (Mulligan et al., 2007). While there have been trials that have

evaluated the effect of micronutrients on appetite, these have been in areas with a low prevalence of HIV (Dossa, Ategbo, van Raaij, de Graaf, & Hautvast, 2001; Dossa, Ategbo, van Raaij, de Graaf, & Hautvast, 2002b; Umeta et al., 2000; UNAIDS, 2007). There are hardly any studies that have assessed the effect of multi-micronutrients on the appetites of HIV-infected children. Multi-micronutrient supplements with and without iron did not improve the appetites (as assessed by *ad libitum* intake of test food) of apparently stable young children from Benin, however the supplements were only given for 14 weeks (Dossa et al., 2001, 2002b). It is possible that the 14-week period of supplementation in stable children was too short. Single micronutrient supplementation has also been used to improve appetite. In a group of stunted Ethiopian children supplementation with zinc only (over six months), significantly reduced the incidence of anorexia, compared to a placebo (Umeta et al., 2000). Iron supplementation (over 12 months) together with anti-helminthic drugs was also reported to have improved the appetites of Zanzibari school children (Stoltzfus et al., 2004). In the Ethiopian and Zanzibari studies poor appetite was assessed by interviewing the mother. Supplementation with iron over 14 weeks improved the appetites (assessed by *ad libitum* intake of test food) of anaemic Kenyan primary school (Lawless et al., 1994). As already noted, these children were anaemic and thus iron is likely to have a noticeable effect compared to placebo.

The results of our study indicate that multi-micronutrient supplementation might be useful in improving appetite in HIV-infected children, and this may improve the nutritional status of these children and thus reduce their susceptibility to infections.

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Chapter 5

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Chapter 5

Chapter 6

Discussion

The studies in this thesis addressed two main aims. The first was to assess the effects of short-term and long-term multi-micronutrient supplementation on episodes of diarrhoea and of respiratory infections in HIV-infected children who were not yet on antiretroviral therapy (ART). The second aim was to assess the impact of long-term multi-micronutrient supplementation on the appetite and growth performance of HIV-infected children who were not yet on ART. A cross -sectional study was performed prior to these intervention studies to compare the appetite, nutritional status and duration of diarrhoea and pneumonia in HIV-infected children (not on ART) with HIV-uninfected children in order to confirm the need for these supplementation studies.

We noted that the appetite and nutritional status of HIV-infected children were poorer than those of uninfected children (Chapter 2). HIV-infected children admitted with diarrhoea or pneumonia were also noted to have a longer duration of hospitalization than those who were not infected (Chapter 2). It was also observed that short-term multi-micronutrient supplementation reduced the duration of acute episodes of diarrhoea and pneumonia (Chapter 3); while long-term supplementation significantly reduced the frequency of episodes of respiratory symptoms and marginally reduced the frequency of episodes of diarrhoea (Chapter 4). Long-term supplementation with the multi-micronutrient supplement also improved the appetite and growth performance of HIV-infected children (Chapters 4 & 5).

In this chapter the following topics will be discussed: methodological issues related to the studies performed, the main findings of these studies, the important role of antiretroviral therapy (ART) in the current treatment of children infected with HIV, the interrelationship between ART and nutritional status and the policy implications arising from the findings of the studies.

Methodological issues related to research performed

This section deals with some of the methodological aspects of the studies that are relevant for the validity of our findings. These aspects relate to the study population, choice of micronutrient supplement and assessment of micronutrient status.

Study population in short and long-term intervention studies

Rationale for enrolment of only HIV-infected children in intervention studies

In the intervention studies that are described in this thesis, the effect of the supplement was assessed only in children who were HIV-infected; HIV-uninfected children were not included. It might be argued that the effects of micronutrient supplementation should have been assessed simultaneously in both HIV-infected and uninfected children, in order to evaluate the magnitude of the effect in HIV-infected children. However, our objective was to assess whether multi-micronutrient supplements were beneficial in HIV-infected children. It was not to compare the magnitude of the effect of the supplements in HIV-infected children with that in uninfected ones. Studies evaluating the effect of multi-micronutrient supplements in HIV-uninfected children from developing countries had been previously conducted by other researchers. These studies indicated that supplementation is beneficial in these children (see Chapter 1). We initially conducted a cross-sectional study that was intended to confirm whether the duration of hospitalization, appetite and nutritional status of HIV-infected children were significantly worse than those of uninfected children. The results of the cross-sectional study suggested that there was a need to assess the effects of multi-micronutrient supplementation specifically in HIV-infected children. We therefore decided to limit the subjects in the intervention studies to HIV-infected children.

Hospital outpatient department based trial versus community based trial

The children who participated in the long-term supplementation study were enrolled from the outpatient department (OPD) of the hospital. Indeed these were children who had previously presented with an illness at the hospital. One might wonder whether children from the OPD are representative of children in their age group in the community. Children who have had previous hospital visits may conceivably have a poorer nutritional status than those who have not required a prior visit to hospital (Lima *et al.* 2004). The results of the study may thus be relevant to children who have already had an illness related to the HIV disease. However, without ART mortality is very high amongst HIV-infected African children; approximately 35% die in the first year and 53% by the second year (Newell *et al.* 2004). There is evidence that a large proportion of ART naïve HIV-infected children who die have had previous hospital admissions (Walker *et al*. 2006). During the period when the study was conducted, ART in the public sector of South Africa was only initiated in hospitals. There are therefore probably not many HIV-infected South African children younger than two years who have not

had a visit to hospital. In view of these facts it is unlikely that there would have been a high number of HIV-infected children without previous hospital visits in a group of children enrolled from the community. We thus consider HIV-infected children enrolled from the OPD as representative of HIV-infected children in their age group in the community.

Appropriate age group for multi-micronutrient supplementation

In the studies of this thesis the supplement was given to HIV-infected children aged 4-24 months at enrolment. One might wonder if this is the appropriate age of giving the supplement in order to ensure the optimal outcomes in HIV-infected children. There are a number of reasons why supplementing to children at this age is a sound idea. HIV-infected children often require hospitalization at this age. South African studies that have assessed the impact of HIV on paediatric hospital admissions have confirmed that the majority of hospitalized HIV-infected children are younger than two years of age (Pillay *et al.* 2001; Meyers *et al.* 2000). It is also estimated that 30% of infants born to HIV-infected mothers get infected; of these 5% are infected in utero, 15% during labour, and 10% in the postpartum period (Merchant and Lala, 2005). It is clear from this that the majority of HIV-infected children are infected during and after birth. The effects of the HIV infection are thus likely to start manifesting in infancy. Therefore giving an intervention to HIV-infected children during infancy seems to be appropriate.

An alternative strategy that has been used is to give micronutrient supplements to pregnant HIV-infected women in order to improve the growth and morbidity of their HIV-infected infants. Studies from Tanzania in particular have shown a beneficial effect in improving the weight-for-length and weight-for-age Z-scores, reducing the risk of acute diarrhoea and improving the CD4 T-lymphocyte counts of children born to these women (Villamor *et al*. 2005; Fawzi *et al.* 2003). In the minority of children who are infected in utero, the mechanisms leading to growth delay are likely to start in utero and to continue after birth (Villamor *et al*. 2005). Indeed HIV-infected pregnant women have a higher risk of delivering low birth weight babies (Fawzi *et al.* 1998). Multi-micronutrient supplementation to pregnant HIV-infected women is therefore a viable alternative to supplementing infants and young children. On the other hand, it might be more practical to give the supplement to HIV-infected infants and young children as they are more likely to present to healthcare facilities at

this age. The administration of supplements to HIV-infected infants and young children when compared to pregnant mothers is probably a more appropriate and practical tool.

Severity of HIV disease in enrolled children

In the studies that are reported in this thesis the clinical stage (WHO, 2005) of the HIV-infected children was not described. It may be considered that the severity of the HIV disease may play a role in children s response to micronutrients, in which case then theseverity of the HIV should be described. The severity of HIV disease can be assessed by clinical or immunological staging (using CD4 lymphocyte counts) (CDC, 1994). Certainly, CD4 lymphocytes were assessed in the children who took part in the long-term supplementation studies. We were thus able to assess the proportion of children with severe immune suppression in these studies (see Chapter 4).

Multi-micronutrient supplementation

Methodological issues relevant to micronutrient supplementation include composition of the supplement and recommended dose of micronutrients.

Composition and dose of the multi-micronutrient supplement

Since multiple micronutrients seem to be of more benefit than a single micronutrient, we decided to use a multi-micronutrient supplement in the short-term and long-term intervention studies. The most prevalent micronutrient deficiencies include iron, zinc, vitamins A, B2, B12, D and E, and these usually occur together (Allen *et al.* 2009). The following micronutrients have also been shown to play a role in the human immune system; zinc, iron, vitamins A, B6, B12, folic acid, C, D and E (Mehta & Fawzi, 2007). As a result we felt that the multi-micronutrient supplement should at least contain the micronutrients that commonly cause deficiencies as well as those that have immunological actions.

The multi-micronutrient supplement that was used in the intervention studies is commercially available and it contains 300 µg retinol, 0.6 mg thiamin, 0.6 mg riboflavin, 8 mg niacin, 0.6 mg pyridoxine, 1 µg cobalamin, 70 µg folic acid, 25 mg ascorbic acid, 5 µg 1,25-dihydrocholecalciferol, 7 mg d,l α-tocopherol, 700 mg copper, 8 mg iron, 30 µg selenium, and 8 mg zinc. The amounts in the supplement were independently tested and confirmed by the Medicines Control Council of South Africa. These amounts are equal to the recommended dietary intake levels for a one year old child. This is line with WHO guidelines

which recommend that micronutrient supplements should be given according to RDA (WHO, 2003). In light of the evidence that high intakes of some micronutrients may produce adverse outcomes in HIV-infected persons (WHO, 2003), it was prudent to supplement at RDA levels as per WHO guidelines. It should be noted that the dose of zinc (8 mg) that was also given in the short-term supplementation study was lower than the recommended 20 mg for acute diarrhoea in children older than six months (WHO & UNICEF, 2004). Still, safe upper limits for micronutrients in children infected with HIV have not been established, thus it was considered that using the RDA levels was appropriate.

Some questions have been raised about the possible pitfalls of supplementing micronutrients to HIV-infected children. These questions relate in particular to zinc, vitamin A and iron, and are partly related to the structure of the virus. The nucleocapsid protein of the human immunodeficiency virus has zinc fingers which have a high affinity for zinc cations and are involved in viral replication. The HIV genome is also known to have a retinoic acid receptor element, thus vitamin A may increase HIV replication (Mehta & Fawzi, 2007). Zinc has also been shown to induce the proliferation of CD4 lymphocytes and activated CD4 lymphocytes are a major target for HIV replication. Vitamin A is known to increase lymphoid cell differentiation, which leads to an increase in CCR5 receptors which are essential for attachment of HIV to lymphocytes (Mehta & Fawzi, 2007). It has also been argued that iron supplementation could have deleterious consequences for HIV-infected people; these adverse effects could include enhanced oxidative stress, increased viral replication and increased mortality (Boelaert *et al*. 1996; Savarino *et al*. 1999). Notwithstanding these concerns, supplementation with the afore-mentioned micronutrients appears to be safe. Zinc supplementation for six months to HIV-infected South African children did not increase plasma HIV viral loads or decrease the percentage of CD4 lymphocytes (Bobat *et al*. 2005). A review by Clark and Semba (2001) indicated that iron supplementation for HIV-infected pregnant women, and for anaemic infants and children as is currently practiced in most developing countries is not contraindicated by available data. Encouragingly, another study noted that while vitamin A supplementation increased the risk of respiratory infection among HIV-uninfected Tanzanian children, it was actually protective among those who were HIV-infected (Fawzi *et al*. 2000).

Adequate assessment of micronutrient status

There is a lack of adequate biomarkers of micronutrient status. Serum concentrations of micronutrients are not considered as accurate indicators of total body micronutrient status.

Zinc status: There are a number of techniques that can be used to assess zinc status; of these the most promising for field application are dietary assessment and plasma/serum zinc concentration measurement (Gibson *et al*. 2008). Despite its limitations, analysis of plasma or serum zinc concentration remains the most practical method currently available to assess zinc status of populations (Hess *et al*. 2007). Consumption of a meal may induce a postprandial reduction in plasma zinc, even if dietary intakes of zinc and tissue reserves are normal (Hambidge *et al*. 1989). To avoid the latter problem it is advisable to obtain fasting serum zinc samples. This was obviously done in the studies reported in this thesis.

Vitamin A status: Vitamin A status can be best expressed in terms of total body reserves of vitamin A, or alternatively of liver concentrations of the vitamin (Tanumihardjo, 2004). In well-nourished persons, the liver contains more than 80% of the total-body stores (Senoo *et al*. 2007). Direct measurement of liver retinol stores by liver biopsy is considered to be the "gold standard", but this is invasive and is thus rarely an option (Tanumihardjo, 2004). While measurement of serum retinol concentration is less sensitive than the techniques used to measure retinol liver stores (Furr *et al*. 1989), it is thought to be adequate for estimating vitamin A status when the levels are very low (< 0.35 μmol/L) or very high (> 3.5 μmol/L) (Furr *et al*. 1989).

Iron status: The measurement of body iron stores through bone marrow staining is considered to be the "gold standard" for determining iron status (Erhardt *et al*. 2004). However, this requires bone marrow aspiration which is an invasive procedure. Serum ferritin and soluble transferrin receptor are considered to be the two best parameters for assessing iron status (Erhardt *et al*. 2004). The high cost and lack of standardisation of the soluble transferrin receptor assay tends to limit its applicability (Yang *et al*. 2008). It is thought that for most programmatically relevant purposes serum ferritin is an effective measure of iron status (Yang *et al*. 2008).

Although the biomarkers for assessing micronutrient status are not adequately developed, the methods used in the studies are suitable for assessing micronutrient status in populations.

Concentrations of serum zinc, retinol and ferritin are adversely affected by inflammation; concentrations of serum zinc and retinol are lower, while those of serum ferritin are high with elevated C-reactive protein (CRP) concentrations (Brown *et al*. 1993; Erhardt *et al*. 2004). Unfortunately, for practical reasons CRP concentrations were not measured in the intervention studies of this thesis. The CRP concentrations would have enhanced the interpretation of the micronutrient concentrations.

Main research outcomes

Differences between HIV-infected and uninfected children (Chapter 2)

We observed that the duration of hospitalization was significantly longer among HIV-infected children than that in those who were uninfected. The nutritional status of children infected with HIV was also shown to be poorer than that of uninfected children. The weight-for-age and length-for-age Z-scores of the children who were HIV-infected with HIV were significantly lower than those of the uninfected children. However, there was no difference in the weight-for-length Z-scores between these two groups of children. HIV-infected children were observed to have significantly poorer appetites than HIV-uninfected children. This was in terms of total amount eaten, duration of eating episodes and eating rate.

The finding of increased duration of hospitalization and a poorer nutritional status among HIV-infected children compared to HIV-uninfected children was consistent with findings of other researchers (Kourtis *et al*. 2006; Heikens *et al*. 2008). The finding of reduced appetite in HIV-infected children as opposed to that in uninfected ones is also in keeping with observations by other authors (Pol *et al*. 2007; Heikens *et al*. 2008). However, other studies had used caregiver reported assessment of appetite, while we used a more objective means of appetite measurement. The comparison with other studies is more extensively reported in Chapter 2.

The duration of hospitalization of HIV-infected was longer and their appetite and nutritional status poorer than that of uninfected children.

We would thus recommend that in communities with high HIV prevalence all children with severe malnutrition should be investigated for HIV infection.

Short-term and long-term multi-micronutrient supplementation and its effects on diarrhoea and respiratory infections in HIV-infected South African children (Chapters 3 and 4)

Our finding was that short-term multi-micronutrient supplementation reduced the duration of episodes of diarrhoea and pneumonia and long-term multi-micronutrient supplementation decreased the frequency of episodes of diarrhoea and respiratory symptoms.

Supplementation with micronutrients during an episode of diarrhoea or respiratory infection has resulted in variable outcomes. Zinc supplementation has been clearly shown to reduce the duration of diarrhoea, but does not seem to be beneficial in acute respiratory infections (Fischer Walker & Black, 2007; Mathew, 2010a) While there is some evidence of the benefits of vitamin A in acute diarrhoea (Mahalanabis & Bhan, 2001), a review by Fischer Walker & Black (2007) suggested that neither vitamin A nor folic acid has an effect on the duration of diarrhoea. Vitamin A does not seem to be useful in non -measles pneumonia, and a systematic review suggested that vitamin A has no benefit in the treatment of childhood pneumonia (Mathew, 2010b). However, multi -micronutrient supplementation reduced the duration of respiratory-related illness in Chinese preschool children compared to children who were only given vitamin A or vitamin A plus iron (Chen *et al*. 2010).

We believe that the decreased duration of hospitalization that we observed is clinically significant. A longer duration of hospitalization has an impact on hospital bed occupancy and may result in the exclusion of other patients who require hospitalization. The risks of acquiring a nosocomial infection are also increased with longer hospitalization. In addition to these, there are financial costs related to hospitalization. The costs of hospitalization include the actual cost of hospital care, as well as the cost of days lost from work by the parents/caregivers and the transport costs of visiting the child in hospital. While calculations of inpatient costs were not done in the current study, it has been estimated that the mean total direct medical cost of hospitalization for a child admitted with diarrhoea at Dr George Mukhari hospital (where the current study was conducted) in 2005 was $1123 (MacIntyre & de Villiers, 2010). We can thus conclude that a reduction of 1.5 days in the duration of hospitalization would result in significant cost savings per patient.

Studies assessing the effect of multi-micronutrient supplementation on the frequency of episodes of diarrhoea and respiratory infections have shown varying results. A study conducted among Peruvian children indicated that while zinc supplementation alone seemed

to reduce the number of episodes of diarrhoea and respiratory illness, a multiple micronutrient supplement (including zinc) actually increased the number of episodes of these diseases (Penny *et al*. 2004). In rural South African children multiple micronutrient supplementation decreased the episodes of diarrhoea and respiratory symptoms, but only among stunted HIV-uninfected children (Chhagan *et al*. 2009). Multi-micronutrient sprinkles (including zinc) also significantly reduced the prevalence of diarrhoea compared to placebo (Sharieff *et al*. 2006). Supplementation with a multi-micronutrient seasoning powder also reduced the incidence of diarrhoea and respiratory related illness in Chinese pre-school children compared to children who were only given vitamin A or vitamin A plus iron (Chen *et al*. 2010).

Our observations were that short-term multi-micronutrient supplementation decreases the duration of hospitalization in HIV-infected children admitted with diarrhoea or with pneumonia. Long-term multi-micronutrient supplementation seems to reduce the number of episodes of diarrhoea and those of respiratory symptoms.

The implementation of multi-micronutrient supplementation in HIV-infected children with an acute episode of diarrhoea or pneumonia should be considered in the clinical setting as this seems to reduce the duration of illness. Long-term multi-micronutrient supplementation in HIV-infected children should also be considered for application as it apparently decreases the frequency of episodes of diarrhoea and respiratory symptoms.

Impact of multi-micronutrient supplementation on growth performance of HIV-infected South African children (Chapter 4)

We noted that while the supplement significantly improved the weight-for-age and weight-for-height Z-scores of these children, it seemed to have no effect on the height-for-age Z-scores. It is possible that supplementation for six months was too short to observe improvement in height. A meta-analysis assessing the effects of vitamin A, iron and multi-micronutrients indicated that neither vitamin A nor iron alone improved either ponderal or linear growth, but multi-micronutrient supplementation improved linear growth (Ramakrishnan *et al*. 2004). A systematic review showed that while the efficacy of multi-micronutrient supplementation varied across trials, there is substantial evidence that growth (in terms of length and weight) is improved, and that the benefits are greater with a multi-micronutrient than with two or fewer micronutrients (Allen *et al*. 2009). Studies that evaluate

the effect of multi-micronutrient supplementation on growth of HIV-infected children are few, and as far as we know no meta-analysis has been performed.

A study such as the one we performed showing improvement in wasting strengthens the case for recommending supplementation to all HIV-infected children.

Effect of multi-micronutrient supplementation on appetite of HIV-infected South African children (Chapter 5)

The multi-micronutrient supplement resulted in a significant improvement in appetite of children when compared with that of children who were on the placebo. This was observed in relation to the absolute amount eaten and amount eaten per kilogram body weight, but not in terms of the duration of eating episodes and the eating rate.

Supplementation with zinc and with iron was noted to improve the appetite (as reported by the mother) of Ethiopian and Zanzibari children respectively (Umeta *et al.* 2000; Stoltzfus *et al*. 2004). However, multivitamin-multimineral supplementation with or without additional iron did not improve the appetite (measured by *ad libitum* intake of test food) of Beninese children (Dossa *et al*. 2001; Dossa *et al*. 2002). It is worth noting that the multivitamin-multimineral supplementation in the Beninese studies was given for six weeks while the zinc and iron supplements were given for six and 12 months respectively. It thus possible that supplementation for a longer period may have yielded positive results.

Our findings were that multi-micronutrient supplementation improves the appetite of HIV-infected children.

We believe that the improvement in the appetite of HIV-infected children that we observed is a meaningful result that would further support a recommendation to give multi-micronutrient supplements to HIV-infected children at a population level.

Pivotal role of antiretroviral therapy in treatment of HIV-infected children

In the first 15 years (1981-1995) of the HIV pandemic, HIV disease was a universally fatal and catastrophic illness (Delaney, 2006). Most HIV programmes at the time focused on prevention of further infections, surveillance of HIV infection and supportive treatment for

the infected people. The supportive treatment was mainly related to treatment of opportunistic infections (UNAIDS, 2000). The development of multi-drug combination therapy for treatment of HIV disease (which became available in 1996) is considered as one of the successes of modern medicine (Delaney, 2006). This combination therapy, where successfully implemented has changed HIV from an almost inevitably fatal disease to a chronic illness (Delaney, 2006). However, ART remained largely unavailable to HIV-infected persons in developing countries until 2002 (WHO, 2002).

In 2000, the United Nations adopted the millennium development goals, which include combating HIV/AIDS, malaria and other infectious diseases (goal number six). One of the targets of this goal was to achieve by 2010, universal access to treatment (ART) for HIV/AIDS for all those who need it (United Nations, 2000). Subsequently WHO released guidelines for scaling up ART in resource limited settings; these guidelines included recommendations for starting ART in infants and children with advanced HIV disease (WHO, 2002). However, subsequent evidence revealed that mortality is high in infancy and that early initiation of ART dramatically reduces this mortality. The guidelines now state that ART should be initiated in all HIV-infected infants, regardless of the CD4 count or whether the infant is sick (WHO, 2010). It is stated that ART should be commenced in children between 12 and 24 months irrespective of clinical and immunological stage, but this is a conditional recommendation; eligibility for ART in children older than 2 years is based on clinical and immunological severity (WHO, 2010).

WHO currently has a number of recommendations relating to nutrition for HIV-infected children, but the quality of evidence supporting a number of them is low. A number of these recommendations state that guidelines that are in place for HIV-uninfected should apply to HIV-infected children as well. These include six monthly high dose vitamin A to children aged 6-59 months, zinc supplementation for acute diarrhoea, and exclusive breastfeeding for 6 months and then continuation until two years and beyond (WHO, 2010). These guidelines also add a conditional recommendation that children who are infected with HIV should receive one RDA of micronutrients daily, and that if this cannot be given through the diet or there is evidence of deficiency, then supplements should be given (WHO, 2010). This is put forward as a conditional recommendation, as there is inadequate and very low quality evidence to support it.

In South Africa, the policies relating to the treatment of HIV infection have not always been in line with WHO guidelines. In 1998, WHO recommended that prevention of mother-to-child transmission should be part of the minimum standard of care for HIV-infected women and that this should include ART to the pregnant mother (WHO, 1998). This was not implemented in South Africa, and it was only after a successful court action by South African NGOs in 2002, that a limited programme of giving ART to pregnant women to reduce HIV transmission to their infants was started. As mentioned before WHO guidelines in 2002 were to give ART to infants and children with advanced HIV disease (WHO, 2002). This again was not implemented and it was only in November 2003 that the South African government published its plan to provide public access to ART in 2004 (many years after the effectiveness of ART in reducing mortality had been reported) (Department of Health, South Africa, 2003). By then some sub-Saharan countries that have less resources than South Africa had already begun to make ART available. The latest South African guidelines (2010) on provision of ART to infants and children are largely in line with WHO recommendations, except that the conditional recommendation of providing ART to children aged 12-24 months regardless of HIV severity is not followed. Similarly, the WHO conditional recommendation of giving multi-micronutrients to all HIV-infected children is also not adhered to (Department of Health, South Africa, 2010).

The clinical efficacy of ART in children in developed countries has been well documented. A study conducted in the United States indicated that the initiation of combination ART was associated with an estimated reduction of 67% in the risk of death (Gortmaker *et al*. 2001). This reduction in risk of death was regardless of age, sex, percentage of CD4 lymphocytes, race and educational level of the parents. Mortality was also markedly reduced in a group of children from California (United States) after the introduction of ART; the hospitalization rate was also markedly reduced (from 6.49 to 0.6 admissions per 100 person-years) (Viani *et al*. 2004) Studies from less developed countries have also showed that excellent clinical response to ART. In a group of South African children, survival after one year post introduction of ART was 84%; this was in spite of the fact that 77% of the children had advanced immune suppression (Eley *et al*. 2006). Another study conducted in Côte d'Ivoire also exhibited a low mortality rate after 42 months of treatment; a good immune

recovery and a sustained viral suppression were also obtained (Rouet *et al*. 2006). Yet the coverage of ART in children in Africa remains low.

Children under one year are among those who are most vulnerable to HIV but are often among the least served (UNAIDS, 2008). Studies from Cambodia and South Africa have indicated that the median age at which ART is commenced is around 6 years (Janssens *et al*. 2007; Reddi *et al*. 2007). A retrospective review of post neonatal mortality in South Africa revealed a peak at 2-3 months of age in HIV-related mortality (Bourne *et al*. 2009). Indeed early initiation of ART has been shown to significantly reduce mortality when compared with ART initiated according to clinical progression of HIV or CD4 counts (Violari *et al*. 2008). Encouragingly the number of children initiated on ART has increased dramatically over the past few years and it is reported that from the end of 2007 to the end of 2008 there was a 39% increase in the number of children initiated on ART (WHO, 2009).

Interrelationship between antiretroviral therapy and nutritional status

It is important to note that the results that reported in this thesis apply to HIV-infected children who are not yet on ART. It is quite conceivable that the response of children who are on ART may not be similar. Nonetheless, failure to address nutritional deficiencies that a re there even in HIV-infected children who are on ART, may limit the potential of ART. Malnutrition at the time of commencing ART has been associated with increased mortality (Paton *et al.* 2006). Indeed, a study in Malawi indicated that adults with severe malnutrition (body mass index < 16.0 kg/m2) had a six times higher risk of dying in the first three months of commencing ART when compared to those with normal nutritional status (Zachariah *et al.* 2006). The association between undernutrition and increased mortality on starting ART has also been seen in children. In a group of HIV-infected Kenyan children who commenced ART, wasting and anaemia were associated with increased early mortality (Wamalwa *et al.* 2010).

Treatment with ART has been shown to h ave beneficial effects on the nutritional status of HIV-infected children. A multi-centre study conducted in the Netherlands showed improvements in weight-for-age and height-for-age Z-scores after the children had received ART for 96 weeks; the improvement was only significant in virologic responders (Verweel *et*

al. 2002). A significant improvement in weight-for-age and height-for-age Z-scores was also observed in Thai children within the first year after ART initiation (Aurpibul *et al.* 2009). Likewise, HIV-infected children from Malawi had significant improvements in WAZ and HAZ, two years after starting ART (Weigel *et al.* 2010). Treatment with ART also significantly improved WAZ and HAZ in Ugandan children within 48 weeks of ART initiation (Musoke *et al.* 2010). It is worth noting that while the mean WAZ returned to -1.5 or greater at 48 weeks among the Thai children, it was not until 144 weeks that the mean HAZ reached -1.5 or greater (Aurpibul *et al.* 2009). Among the Malawian children neither the median WAZ nor the median HAZ reached normal values after two years on ART among children who were underweight or stunted at baseline (Weigel *et al.* 2010).

It seems that ART may also influence the concentrations of some micronutrients. A systematic review of observational studies of micronutrients in HIV-infected persons receiving ART suggested that some micronutrients may increase after ART initiation (Drain *et al.* 2007). Treatment with ART increased the concentrations of α-carotene, β-carotene, α-tocopherol, vitamin B-12 and folate, but not of vitamin A, selenium or zinc (Drain *et al.* 2007). Conversely, a study that compared serum zinc concentrations of HIV-infected children on ART with that of HIV-infected children who were not on ART revealed that children on ART were significantly less likely to be zinc deficient (Ndeezi *et al.* 2010).

Micronutrient supplementation may be of benefit to HIV-infected individuals on ART as well. Zinc supplementation for 18 months reduced the rate of diarrhoea and the likelihood of immunological failure in zinc deficient HIV-infected adults (Baum *et al*. 2010). Supplementation with multi-micronutrients improved the CD4 counts of adults infected with HIV who were on ART (Kaiser *et al*. 2006). On the other hand some studies have not shown an effect of micronutrient supplementation on viral load or CD4 counts (Semba *et al*. 2007; Batterham *et al*. 2001).

It is important to note that there are adverse nutritional and metabolic consequences of ART use; these include dyslipidaemia (elevated circulating concentrations of total cholesterol and triglycerides), lipodystrophy, glucose intolerance and insulin resistance (Raiten *et al.* 2005). Other nutritional abnormalities include lactic acidaemia, osteopaenia and osteoporosis (Raiten *et al.* 2005).

Treatment of dyslipidaemia as a consequence of ART is the same as when it results from other causes. The treatment includes a low-fat diet, exercise and medical therapy (fibrates and statins) when required (Dubé *et al.* 2003). There is also some evidence that micronutrients may be of some benefit in ART related dyslipidaemia. Extended release niacin was noted to significantly improve total cholesterol (decreased), triglycerides (decreased), VLDL (decreased) and HDL (increased) in non-diabetic HIV-infected adult males who were on ART and had confirmed dyslipidaemia (Dubé *et al.* 2006). An earlier trial had also shown that extended release niacin had a beneficial effect in HIV-infected adults on ART with dyslipidaemia (Gerber *et al.* 2004). Both these trials indicated that extended release niacin was safe in these patients, especially in relation to concerns about insulin resistance (Dubé *et al.* 2006; Gerber *et al.* 2004). Thiamine and riboflavin may be of benefit in treating the lactic acidosis that may be caused by the nucleoside reverse transcriptase class of ART; some case report studies on HIV-infected adults have also suggested this beneficial effect (Dalton & Rahimi, 2001; Arici *et al.* 2001). There seems to be evidence of the contribution of ART to the reduced bone mineral density that is observed in HIV-infected patients; the role of vitamin D and calcium supplementation needs further investigation (Stone *et al.* 2010).

Implications for Public Health Policies arising from research described in thesis

Current WHO/UNICEF recommendations for the treatment of acute diarrhoea are for the provision of 10 mg (in children younger than 6 months) or 20 mg of zinc (in children who are 6 months or older) daily for 14 days (WHO and UNICEF, 2004). This dose has been worked out for HIV-uninfected children, and it has not been demonstrated to be safe in HIV-infected children. However, it is thought that short-term zinc supplementation is unlikely to have deleterious effects. There are no current recommendations relating to micronutrient supplementation in children with acute non-measles pneumonia, outside the neonatal period. We showed that multi-micronutrient supplementation reduces the duration of diarrhoea and pneumonia in HIV-infected children (Chapter 3). While further studies may be needed to confirm these findings, serious consideration should be given to supplementing HIV-infected children suffering from acute diarrhoea or pneumonia with multi-micronutrients.

Our finding that long-term multi-micronutrient supplementation improves appetite, reduces wasting and decreases the number of episodes of diarrhoea and respiratory symptoms serves to strengthen the current conditional recommendation by WHO that HIV-infected children should receive micronutrients at RDA levels daily. Relationships that are observed in randomised controlled trials provide a high quality form of evidence. Studies like the ones we performed may add to the data that the WHO requires to strengthen the level of recommendation from conditional into strong.

Recommendations for future research

As noted previously ART does improve nutritional status, but improvement in some of the parameters like height may take as long as two years. Provision of ART seems to have a variable effect on micronutrients, and deficiency of micronutrients increases the risk of diarrhoea and respiratory infections (see Chapter 1). Whether the addition of multi-micronutrient supplements to ART results in greater and quicker improvement in growth and reduction in episodes of diarrhoea and respiratory infections in HIV-infected children who are on ART needs to be assessed.

There is some evidence (as mentioned above) that micronutrients may be helpful in improving the immunological status of HIV-infected adults on ART. Studies need to be conducted to see if these benefits extend to HIV-infected children on ART.

The role of micronutrient supplements in alleviating the adverse nutritional and metabolic effects of ART in children infected with HIV also needs elucidation.

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Summary

Summary

The nutritional status of HIV-infected children as established by weight, height and micronutrient status has been observed to be poor. Diarrhoea and respiratory infections have been shown to be more common and more severe in these children than in HIV-uninfected children.

Deficiencies of micronutrients are associated with poor growth and immune suppression. The suppressed immunity results in increased risk of infections, among others, diarrhoea and respiratory infections. There is evidence that micronutrient supplementation may improve growth and may reduce infections in children who are not HIV-infected. Micronutrient deficiencies seldom occur as a deficiency of a single micronutrient, but rather as a simultaneous deficiency of several micronutrients: a multi-micronutrient deficiency. Supplementation with a multi-micronutrient supplement should therefore be advised above supplementation with a single micronutrient.

The studies that are reported in this thesis aimed to assess the short-term and long-term effects of multi-micronutrient supplementation on diarrhoea and respiratory infections, and on appetite and growth, of HIV-infected children who are not yet on antiretroviral therapy (ART). We have conducted four studies.

The first study performed was a cross-sectional study in which the duration of hospitalization, nutritional status and appetite of HIV-infected children hospitalized with diarrhoea or pneumonia were compared with those of HIV-uninfected children (Chapter 2). In total 192 children aged 4-24 months were enrolled within 24 hours of admission and their HIV status was determined and their weights and lengths were measured. On discharge the duration of hospitalization was recorded and they returned to hospital 4-8 weeks later for appetite testing and assessment of serum micronutrient status. Appetite was tested by *ad libitum* consumption of a commercial baby cereal on three separate days using a standardized protocol. HIV-infected children were observed to be more wasted but not more stunted than those who were uninfected. The duration of hospitalization was 52% longer in HIV-infected children compared with those who were uninfected. Serum zinc, iron and transferrin concentrations and haemoglobin levels were significantly lower in HIV-infected children compared with those who were not infected. The appetite of HIV-infected children as established as amount of test food eaten and as eating rate, was also poor compared with that

in uninfected children. In literature much has been reported on the effects of micronutrient supplementation in HIV-uninfected children, but hardly on effects in HIV-infected children. Since we observed in our study that the starting position with respect to hospitalization duration, nutritional status and appetite was worse in HIV-infected children compared with HIV-uninfected children, we decided to investigate the effects of multi-micronutrient supplementation in HIV-infected children. The supplement used in the short-term and long-term intervention studies contained vitamins A, B complex, C, D, E and folic acid, and the minerals iron, copper, selenium and zinc, at amounts based on recommended daily allowance for a 1 year old child. In the short-term intervention study the supplement was given daily from enrolment until discharge from the hospital, while in the long-term studies it was given daily for 6 months.

In chapter 3 we reported on a short-tem intervention study in which HIV-infected children aged 4-24 months hospitalized with diarrhoea or with pneumonia were randomized to receive either a multi-micronutrient supplement or a placebo until discharge from hospital. A total of 120 children were enrolled within 24 hours of hospitalization (70 hospitalized with pneumonia and 50 with diarrhoea). Supplementation reduced the duration of hospitalization among children admitted with diarrhoea and with pneumonia by 1.6 and 1.9 days respectively. The duration of hospitalization on the supplement was 7.3 ± 3.5 days and on the placebo it was 9.0 ± 4.9 days (P <0.05). We thus conclude that multi-micronutrient supplementation may decrease the duration of hospitalization by 20%.

Subsequently the effects of long-term multi-micronutrient supplementation were evaluated in a randomized trial with 201 HIV-infected children aged 4-24 months (Chapter 4). The children received either supplements or placebos daily for 6 months. The children were monitored for symptoms of respiratory infection (cough and fever) or for diarrhoea twice a week and their weights and heights were measured monthly. CD4 lymphocyte counts were measured on enrolment and at 3 and 6 months. Out of the 201 children who were originally enrolled, 121 completed the study (40% dropout). Among the children who dropped out mortality was 54%. The children who dropped out were more wasted (-1.46 ± -1.31) vs (-1.30 ± 1.26) and stunted (-2.54 ± 1.57) vs (-1.27 ± 1.61) and had lower CD4 counts (17.7 ± 12.6) vs (26.6 ± 12.2) when compared with those who completed the study. Supplementation improved the weight-for-age (0.39 ± 1.07 vs -0.19 ± 1.10) ($p<0.05$) and weight-for-height Z-

scores (0.40 ± 1.22 vs -0.04 ± 1.40 ($p<0.05$) when compared with children who received a placebo, but supplementation had no effect on the height-for-age Z-scores (0.09 ± 0.95 vs 0.03 ± 0.88). The supplement did not result in improvement in the CD4 lymphocyte counts over the 6-month period in the supplement (-0.64 ± 8.3) and placebo groups (-1.44 ± 7.5), but it did result in a reduction in the number of episodes of symptoms of respiratory infection (0.66 ± 0.51 vs (0.66 ± 0.51) and of diarrhoea (0.25 ± 0.31 vs 0.36 ± 0.36; $p=0.09$). We thus conclude that long-term (six months) multi-micronutrient supplementation will result in an improvement of body weight and in less episodes of respiratory infection and diarrhoea. The long-term effects of multi-micronutrient supplementation on appetite were assessed in 140 HIV-infected children whose ages were 6-24 months (Chapter 5). The children had appetite tests conducted at enrolment, and at 3 and 6 months. Appetite was measured as in the cross-sectional study as *ad libitum* intake of a commercial baby cereal after an overnight fast using a standardized protocol. A total of 99 of these children (50 on supplements and 49 on placebos) completed the study. The change in appetite over six months was better in children who received the supplement (4.7 ± 14.7 (g of test food eaten per kg body weight)) compared with children who received the placebo (-1.4 ± 15.1 g/kg body weight). Our conclusion is that long-term supplementation with multi-micronutrients will improve the appetite of HIV-infected children.

Our findings from the intervention studies were obtained in HIV-infected children who are not yet on ART. The importance of ART in the treatment of HIV-infected children cannot be overemphasised. However, in spite of the scaling up of access to ART, less than 50% of the estimated 2.3 million HIV-infected sub-Saharan children currently receive ART. The benefits of multi-micronutrient supplementation as described in our studies remain therefore relevant for a large number of children.

Samenvatting

Samenvatting (Summary in Dutch)

De voedingstoestand van hiv-geïnfecteerde kinderen zoals vastgesteld op basis van hun gewicht, lengte en micronutriëntenstatus, is slecht te noemen. Diarree en luchtweginfecties komen in deze kinderen vaker en in ernstiger mate voor dan in kinderen die niet hiv-geïnfecteerd zijn.

Micronutriëntdeficiënties zijn geassocieerd met slechte groei en met onderdrukking van het immuunsysteem. Onderdrukking van het immuunsysteem resulteert in een toegenomen risico op infecties, dus ook op diarree en luchtweginfecties. Aangetoond is dat micronutriëntsuppletie bij kinderen die niet hiv-geïnfecteerd zijn de groei kan verbeteren en het aantal infecties kan terugbrengen. Micronutriëntdeficiënties komen zelden voor als een deficiëntie aan één enkel micronutriënt, in het algemeen komen meerdere deficiënties simultaan voor en spreekt men van multi-micronutrëntdeficiënties. Het ligt dus meer voor de hand te suppleren met een multi-micronutriëntsupplement dan met één enkel micronutriënt.

De studies waarover in dit proefschrift gerapporteerd wordt hadden als doel de korte-termijn en langetermijn effecten vast te stellen van een multi-micronutriëntsupplement op diarree en luchtweginfecties, en op eetlust en groei, bij hiv-geïnfecteerde kinderen die geen antiretrovirale behandeling krijgen. We hebben vier studies uitgevoerd.

De eerste studie was een cross-sectioneel onderzoek waarin de hospitalisatieduur, de voedingstoestand en de eetlust van hiv-geïnfecteerde kinderen vergeleken werd met die van niet-hiv-geïnfecteerde kinderen (Chapter 2). De kinderen waren opgenomen in het ziekenhuis vanwege diarree of luchtweginfecties. In totaal namen 192 kinderen in de leeftijd van 4-24 maanden deel aan het onderzoek. Binnen 24 uur na opname in het ziekenhuis werd hun hiv-status vastgesteld en hun lengte en gewicht gemeten. Bij ontslag uit het ziekenhuis werd de hospitalisatieduur genoteerd en 4-8 weken later kwamen de kinderen terug in het ziekenhuis voor een eetlusttest en voor vaststelling van de micronutriëntenstatus in serum. De eetlust werd vastgesteld door het meten van de *ad libitum* consumptie van een commercieel verkrijgbare kindervoeding op drie verschillende dagen en volgens een gestandaardiseerd protocol. De hiv-geïnfecteerde kinderen waren magerder ("more wasted") maar niet korter ("not more stunted") dan niet-hiv-geïnfecteerde kinderen. De hospitalisatieduur van hiv-geïnfecteerde kinderen was 52% langer, en hun zink, ijzer en transferrine concentraties in

serum alsmede hun hemoglobinespiegels waren significant lager. Ook de eetlust van hiv-geïnfecteerde kinderen uitgedrukt als hoeveelheid gegeten testvoeding en als eetsnelheid was lager dan die bij niet-hiv-geïnfecteerde kinderen. In de literatuur is veel gerapporteerd over de effecten van micronutriëntsuppletie bij niet-hiv-geïnfecteerde kinderen, maar nauwelijks over effecten bij hiv-geïnfecteerde kinderen. Aangezien wij in onze studie vonden dat de uitgangssituatie wat betreft hospitalisatieduur, voedingstoestand en eetlust bij hiv-geïnfecteerde kinderen duidelijk minder gunstig is dan bij niet-hiv-geïnfecteerde kinderen, besloten we de effecten van multi-micronutriëntsuppletie te onderzoeken bij hiv-geïnfecteerde kinderen.

Het supplement dat in de korte-termijn en langetermijn interventiestudies gebruikt is bevatte de vitamines A, B-complex, C, D, E, en foliumzuur, en de mineralen ijzer, koper, selenium en zink, in hoeveelheden die gebaseerd zijn op de dagelijkse aanbevolen hoeveelheden voor een kind van 1 jaar oud. In de korte-termijn studie werd het supplement tijdens de hospitalisatie dagelijks verstrekt tussen de opname in het ziekenhuis en het ontslag. In de langetermijn studies werd het gedurende zes maanden dagelijks verstrekt.

In Chapter 3 wordt gerapporteerd over de korte-termijn interventiestudie bij hiv-geïnfecteerde kinderen van 4-24 maanden oud die in het ziekenhuis opgenomen waren met diarree of luchtweginfecties. Deze kinderen kregen ad random óf dagelijks een multi-micronutriëntsupplement, óf een placebo. In totaal werden 120 kinderen binnen 24 uur na opname in het ziekenhuis gerekruteerd: 70 waren opgenomen met luchtweginfecties en 50 met diarree. Suppletie verminderde de hospitalisatieduur van kinderen opgenomen voor diarree en voor luchtweginfecties met respectievelijk 1,6 en 1,9 dagen. De hospitalisatieduur van de kinderen op het supplement was 7,3 ± 3,5 dagen en van de kinderen op de placebo 9,0 ± 4,9 dagen ($p<0,05$). We kunnen dus concluderen dat multi-micronutriëntsuppletie de hospitalisatieduur van hiv-geïnfecteerde kinderen met circa 20% kan verlagen.

Vervolgens zijn in een interventiestudie de effecten van langetermijn multi-micronutriëntsuppletie geëvalueerd bij 201 hiv-geïnfecteerde kinderen in de leeftijd van 4-24 maanden (Chapter 4). De kinderen kregen ad random óf gedurende 6 maanden dagelijks het supplement óf gedurende 6 maanden dagelijks de placebo. De kinderen werden twee keer per week onderzocht op luchtweginfectie symptomen (hoesten en koorts) en op diarree. Hun gewicht en lengte werd maandelijks gemeten. CD4 lymfocyten werden bepaald bij aanvang

van de studie en na 3 en 6 maanden. Van de 201 gerekruteerde kinderen hebben 121 kinderen de studie afgemaakt (40% uitval). De sterfte onder de kinderen die uitvielen bedroeg 54%. De kinderen die uitvielen waren bij aanvang magerder ("more wasted") (-1,46 ± 1,31 vs -1,30 ± 1,26) en korter ("more stunted") (-2,54 ± 1,57 vs -1,27 ± 1,61)) en hadden minder CD4 cellen (17,7 ± 12,6 vs 26,6 ± 12,2) dan de kinderen die de studie voltooiden. Vergeleken met de kinderen die de placebo kregen leidde suppletie tot een verbetering van de gewicht-voor-leeftijd (0,39 ± 1,07 vs -0,19 ± 1,10) ($p<0,.05$) en de gewicht-voor-lengte Z-scores (0,40 ± 1,22 vs -0,04 ± 1,40 ($p<0,05$), maar het had geen effect op de lengte-voor-leeftijd Z-scores (0,09 ± 0,95 vs 0,03 ± 0,88). Het supplement leidde over de 6 maanden van interventie niet tot een verbetering in het aantal CD4 cellen (-0,64 ± 8,3 in de supplementgroep vs -1,44 ± 7,5 in de placebogroep), maar wel tot een vermindering van het aantal episodes per maand van luchtweginfectie symptomen (0,66 ± 0,51 vs 1,01 ± 0,67; $p<0,05$) en van diarree (0,25 ± 0,31 vs 0,36 ± 0,36; $p=0,09$). We concluderen dan ook dat langetermijn multi-micronutriëntsuppletie bij hiv-geïnfecteerde kinderen zal leiden tot gewichtsverbetering en tot minder episodes van luchtweginfecties en diarree.

De langetermijn effecten van multi-micronutriëntsuppletie op eetlust werden vastgesteld bij 140 hiv-geïnfecteerde kinderen in de leeftijd van 6-24 maanden (Chapter 5). De kinderen ondergingen een eetlusttest bij aanvang van de studie en na 3 en 6 maanden. De eetlust werd vastgesteld zoals in de cross-sectionele studie door het meten van de *ad libitum* consumptie van een commercieel verkrijgbare kindervoeding op drie verschillende dagen en volgens een gestandaardiseerd protocol. In totaal voltooiden 99 kinderen de studie, 50 uit de supplement groep en 49 uit de placebogroep. De verandering in consumptie tijdens de 6 maanden durende interventie was in de supplementgroep gunstiger dan in de placebogroep (+4,7 ± 14,7 g per kg lichaamsgewicht versus -1,4 ± 15,1 g/kg lichaamsgewicht). We concluderen dat langetermijn multi-micronutriëntsuppletie bij hiv-geïnfecteerde kinderen zal leiden tot een verbetering van hun eetlust.

Onze bevindingen met de interventiestudies betreffen hiv-geïnfecteerde kinderen die nog geen antivirale middelen toegediend kregen. Het belang van antiretrovirale behandeling van hiv-geïnfecteerde kinderen kan niet genoeg onderstreept worden en alle hiv-geïnfecteerde kinderen zouden deze middelen moeten krijgen. Echter, ondanks de toegenomen verspreiding van antiretrovirale middelen krijgt momenteel minder dan 50% van de geschatte 2,3 miljoen

hiv-geïnfecteerde kinderen in subsaharaans Afrika antiretrovirale behandeling. De in onze studies waargenomen verbeteringen in voedingstoestand en gezondheidstoestand met multi-micronutriëntsuppletie zijn dus nog steeds relevant voor een grote groep kinderen.

Samenvatting

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About the author

Curriculum vitae

Siyazi Mda was born on February 1, 1963, in Cape Town, South Africa. He obtained the Bachelor of Medicine and Bachelor of Surgery (MBChB) degree at the University of Natal in South Africa, in 1986. After completing his internship at GaRankuwa hospital in 1987, he worked as a general medical practitioner in Port Elizabeth, South Africa. In 1991, he started his training in Paediatrics at the University of Witwatersrand, and subsequently transferred to the Medical University of Southern Africa (Medunsa) in 1995 where he completed his paediatric training and subsequently obtained his Master of Medicine in Paediatrics. He has worked as a lecturer in the Department of Paediatrics and Child Health at Medunsa and as a consultant in paediatrics at GaRankuwa hospital (now Dr George Mukhari hospital) since 1996. In 2007 he was appointed principal paediatrician and senior lecturer at the Department of Paediatrics and Child Health at Dr George Mukhari hospital and at the Medunsa campus of the University of Limpopo, respectively.

He started work on his PhD at the Division of Human Nutrition at Wageningen University in November 2003. The data belonging to the research presented in this thesis was collected between August 2004 and November 2006, in South Africa.

He received the Nestlé Nutrition Institute in Africa award for the best scientific publication in 2010 for nutrition research conducted in Africa for the article entitled "Short-term micronutrient supplementation reduces the duration of pneumonia in HIV-infected children" which is published in *Journal of Nutrition 2010; 140:969-974.*

List of publications

Original research papers

Mda S, van Raaij JM, MacIntyre UE, de Villiers FP, Kok FJ. Improved appetite after multi-micronutrient supplementation for six months in HIV-infected children. *Appetite 2010; 54:150-155.*

Mda S, van Raaij JM, de Villiers FP, MacIntyre UE, Kok FJ. Short-term micronutrient supplementation reduces the duration of pneumonia in HIV-infected children. *Journal of Nutrition 2010; 140:969-974.*

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Mda S, Raaij JMA van, MacIntyre UE, Villiers FPR de, Graaf C de, Kok FJ. Poor appetite in HIV-infected South African children. Abstracts 18th International Congress of Nutrition, September 19-23, 2005, Durban, South Africa. *South African Journal of Clinical Nutrition* 2005; 18(1): p 248 (ABSTRACT 2.5.26).

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Educational program

Discipline specific activities

Nutritional and Lifestyle Epidemiology course, VLAG, Wageningen, 2003

Management of HIV course, South African Medical Association, 2004

Human Nutrition course: Self-study with assessment by Dr van Raaij, 2004

Statistics in Nutrition and SPSS course: Self-study with assessment by Dr van Raaij, 2004

International Congress of Nutrition, Durban, South Africa, *poster presentation*, 2005

South African Nutrition Congress, Port Elizabeth, South Africa, 2006

South African Nutrition Congress, Pretoria, South Africa, *oral presentation* 2008

International Congress of Nutrition, Bangkok, Thailand, *oral presentation*, 2009

International Pediatric Congress, Johannesburg, South Africa, *poster presentation*, 2005

General courses

Research methodology course, Medical University of Southern Africa, South Africa, 2003

Good practice in clinical research course, Pretoria, South Africa, 2006

Optional courses and activities

Journal club discussion group, Department of Paediatrics, Medical University of Southern Africa, South Africa, (weekly meetings) 2004 -2010

About the author

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