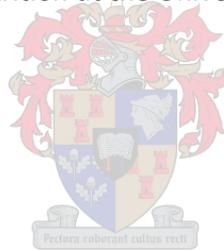


The Effect of an Enriched Nutritional Supplement on Growth and Inflammatory markers in Underweight HIV-positive children aged 24-72 months

by

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification

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ABSTRACT

BACKGROUND: Infection with the human immuno-deficiency virus (HIV) causes immune impairment which leads to malnutrition. Malnutrition worsens the effects of HIV, resulting in faster progression to acquired immune-deficiency syndrome (AIDS). The combination of malnutrition and HIV in children negatively impacts their growth and immune status. Addressing malnutrition in HIV-positive individuals can potentially result in a strengthened immune system which is better able to cope with opportunistic infections. This thesis explored whether a cohort of HIV-positive children in a child outpatient healthcare setting and receiving nutritional supplementation showed associated changes in defined anthropometric indices (height, weight), and in defined immune and inflammatory markers.

DESIGN: The thesis describes a sub-study within a successfully completed large randomized controlled clinical trial. The latter compared catch-up weight in HIV positive children receiving a complete nutritional supplement and were randomized to two groups. One group consumed supplement A (complete supplement) and the other consumed supplement B (complete supplement with added functional nutrients). The sub-study analysed data on the whole cohort and also conducted a sub-group analysis to determine bio-equivalence of the two supplements.

METHODOLOGY: Study participants were recruited if they were HIV-positive, between the ages of 24-72 months and met the trial's pre-determined inclusion criteria. Participants were recruited from Anti-retroviral treatment (ART) clinics within the Cape Town Metropole area. Anthropometric data (weight; height) was collected at baseline and at each of the six follow-up visits. Venous blood was drawn to analyse biomedical parameters [C-reactive protein (CRP); calprotectin; Immunoglobulin A (IgA)]. Anthropometric data was analysed using the World Health Organisation (WHO) Anthro Plus program and SAS General linear models (GLM Procedure) was used to calculate change overtime. Biomedical raw data was exported to STATA and statistical significance was calculated using the Related-Samples Wilcoxon Signed Rank test.

RESULTS: The total number of children included in the study was 138 (64 boys; 74 girls). The cohort comprised of 69 (50%) children aged 2-3 years and 69 (50%) aged 4-6 years. The addition of the complete nutritional supplement, in its two variants, in the cohort increased mean energy intake by 1000kJ for both age-categories. For the 2, 4 and 5 year old children, weight and height gain from baseline to visit 6 was significant ($p < 0.000$;

$p < 0.001$; $p < 0.000$; $p < 0.000$; $p < 0.004$; $p < 0.000$). Weight-for-age z-scores (WAZ) improved significantly for the 2-year old children ($p < 0.0082$) only.

CRP and calprotectin levels of the cohort showed a significant reduction to within normal ranges ($p < 0.002$; $p < 0.005$). Immunoglobulin A (IgA) was substantially reduced ($p < 0.003$) close to the normal range. Children receiving supplement B had a significant improvement in the $< -3SD$ height-for-age z-scores (HAZ) in comparison to group A ($p < 0.038$). Children $< 5^{\text{th}}$ percentile for weight-for-age (WFA) and height-for-age (HFA) showed a significant improvement in IgA levels compared to group A ($p < 0.018$). Children receiving supplement A, who were $> 5^{\text{th}}$ percentile for WFA, showed a significant improvement in CRP levels compared to children receiving supplement B.

CONCLUSION: The findings of this study showed a positive effect on energy intake by consuming a balanced nutritional supplement, irrespective of composition, and on the growth and immune markers of HIV-positive, underweight children within in an outpatient setting. The addition of specific functional nutrients was found to be safe but efficacy needs to be further investigated.

OPSOMMING

AGTERGROND: Infeksie met die menslike immuuniteitsgebreksvirus (MIV) veroorsaak immuun-inkorting wat lei tot wanvoeding. Wanvoeding vererger die effek van MIV wat lei tot vinniger vordering na vervorwe immuuniteitsgebrek-sindroom (VIGS). Die kombinasie van wanvoeding en MIV het 'n negatiewe impak op die groei- en immuun-status van kinders. Die aanspreek van wanvoeding in HIV-positiewe individue kan moontlik lei tot 'n versterking van die immuunstelsel wat opportunistiese infeksies beter kan hanteer. Hierdie tesis het ten doel gehad om te ondersoek of 'n groep MIV-positiewe kinders, in 'n kinder-buitepasiënt gesondheidsorg-instelling, wat voedingsaanvullings ontvang het, ooreenstemmende veranderinge in die voorkoms van gedefinieerde antropometriese indekse (lengte, gewig) en in gedefinieerde immuun- en inflammasie merkers getoon het.

ONTWERP: Die tesis beskryf 'n sub-studie binne 'n suksesvol voltooide groot ewekansige behoorde kliniese proefneming. Laasgenoemde het die inhaal-gewig in MIV-positiewe kinders wat 'n volledige voedingsaanvulling ontvang het vergelyk en was lukraak toegewys in twee groepe. Een groep het aanvulling A (volledige aanvulling) ingeneem en die ander aanvulling B (volledige aanvulling met meer funksionele voedingstowwe). Die sub-studie het data ontleed van die hele kohort en het ook 'n sub-groep analise ingesluit om bio-ekwivalensie van die twee aanvullings te bepaal.

METODE: Deelnemers was ingesluit indien hul MIV-positief was, tussen die ouderdomme van 24-72 maande en voldoen het aan voorafbepaalde kriteria vir insluiting in die studie. Deelnemers was gewerf uit anti-retrovirale terapie (ART) klinieke binne die Kaapse Metropool area. Antropometriese data (gewig; lengte) is tydens die basislyn versamel asook tydens elk van die ses opvolgbesoeke. Veneuse bloed was getrek om biomediese parameters te ontleed (C-reaktiewe proteïen [CRP]; calprotectin; Immunoglobulien A [IgA]). Antropometriese data was ontleed met behulp van die WGO Anthro Plus program en SAS algemene lineêre modelle (GLM Procedure) is gebruik om verandering met verloop van tyd te bereken. Biomediese rou data is uitgevoer na STATA en statistiese betekenisvolheid was bereken deur die Verwante-Monsters Wilcoxon rangordetoets.

RESULTATE: Die totale aantal kinders, ingesluit in die studie, was 138 (64 seuns, 74 dogters). Die groep het bestaan uit 69 (50%) kinders van 2-3 jaar en 69 (50%) van 4-6 jaar oud. Die byvoeging van die voedingsaanvulling in die groep het die gemiddelde energie-inname met 1000kJ verhoog vir beide ouderdomskategorieë. Die gewig en lengte toename

vir die 2, 4 en 5 jaar oue kinders vanaf die basislyn tot die 6de besoek was betekenisvol ($p < 0,000$; $p < 0,001$; $p < 0,000$; $p < 0,000$; $p < 0,004$; $p < 0,000$). Gewig vir ouderdom z-tellings het aansienlik verbeter slegs vir die 2-jarige kinders ($p < 0,0082$).

CRP en calprotectin het beduidend verlaag tot binne normale waardes ($p < 0,002$; $p < 0,005$). Immunoglobulien A het beduidend verlaag ($p < 0,003$) tot naby aan normale reikwydtes. Kinders wat supplement B ontvang het, het 'n beduidende verbetering in die $< -3SD$ HAZ groep in vergelyking met 'n groep A getoon ($p < 0,038$). Kinders $< 5^{\text{de}}$ persentiel vir gewig-vir-ouderdom (GVO) en lengte-vir-ouderdom (LVO) het 'n beduidende verbetering in IgA vlakke in vergeleke met groep A ($p < 0,018$) getoon. Kinders wat supplement A ontvang het en $> 5^{\text{de}}$ persentiel vir GVO was, het 'n beduidende verbetering in CRP vlakke getoon in vergelyking met kinders wat supplement B ontvang het.

GEVOLGTREKING: Die bevindinge van hierdie studie dui die positiewe gevolge van gebalanseerde voedingsaanvullings, ongeag die samestelling daarvan, op die groei - en immuun-merkers van MIV-positiewe, ondergewig kinders in 'n buitepasiënt omgewing aan. Die byvoeging van 'n spesifieke funksionele voedingstowwe was veilig bevind, maar doeltreffendheid moet verder ondersoek word.

DEDICATION

To my Mom and Dad for always pushing me to be the best I can be.

And

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Love you all dearly!

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Contributions by student, statistician and supervisors

The Principle Investigator (Prof D Labadarios) developed the idea and the study protocol for the main study. The student provided input to inclusion criteria and operational aspects of the main study. The student developed the idea and study protocol for this thesis.

The student undertook data collection and data cleaning for the data analysis with the assistance of two statisticians (Prof H Nel and Mrs T Esterhuizen), interpreted the data and drafted the thesis. Prof D Labadarios provided input at all stages and revised the protocol and thesis. Dr LM du Plessis provided input and revised the protocol and thesis.

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GLOSSARY/ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
BMI	Body mass index
CD4	CD4 is a glycoprotein found on the surface of immune cells.
CDC	Centre for Disease Control (USA)
CRF	Case Report Form
CRP	C-reactive protein, an acute phase protein.
DHA	Docosahexaenoic acid. An omega-3 fatty acid
DRI	Dietary Reference Intake
EAR	Estimated average requirement
EPA	Eicosapentaenoic acid. An omega-3 fatty acid
FAO	Food and agricultural organization
FFQ	Food frequency questionnaire
GIT	Gastrointestinal tract
HAART	Highly Active antiretroviral therapy
HAZ	Height-for-age z-score
HIV	Human immunodeficiency virus, the cause of AIDS
IgA	Immunoglobulin A
ISAK	International Society for the Advancement of Kinanthropometry
MGRS	Multicentre growth reference study
MMS	Multiple micronutrient supplementation
MRC	Medical Research Council (South Africa)
n	Sample size
NCHS	National Centre for Health Statistics (USA)
NEC	Necrotising enterocolitis
NHLS	National Health Laboratory Services (South Africa)
NRC	Nestle Research Centre
NTP	Nutrition Therapeutic Program
PMTCT	Prevention of Mother-to-child transmission
PUFA	Poly unsaturated fatty acids
RDA	Recommended daily allowance
RNI	Recommended nutrient intake
RTC	Randomized control trial
SAM	Severe malnutrition

SCFA	Short chain Fatty acids
TB	Tuberculosis
UNAIDS	United Nations Programme on HIV and AIDS
UNICEF	United Nations Children's Fund
WAZ	Weight-for-age z-score
WHO	World Health Organisation

CHAPTER 1: LITERATURE OVERVIEW

1.1 GLOBAL AND NATIONAL PREVALENCE OF HIV AND MALNUTRITION

UNAIDS estimated that globally, in 2014, 36.9 million adults and children were living with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS). In 2014, a total of 2 million adults and children globally were newly infected with HIV. It was further estimated that 1.2 million people died of AIDS-related causes in 2014. Globally, there are currently 2.6 million children under the age of 15 years infected with HIV^(1,2). The new infections in children originate from HIV transmission during pregnancy, childbirth or breastfeeding. Globally, women account for 51% of all adults living with HIV/AIDS^(1,3).

The estimates for sub-Saharan Africa for people living with HIV are 25.8 million and 2 million for newly infected individuals. In 2014, sub-Saharan Africa was the region that carried the highest HIV burden globally. Of all the people living with HIV in sub-Saharan Africa, 59% are women. More than 1 million women who were living with HIV in 2014 gave birth. In 2014, 2.3 million children, 0 to 15 years, were living with HIV^(1,2,4).

UNICEF reported that 5.9 million children under the age of 5 years died in 2014. Half of these deaths were attributable to undernutrition. Wasting on a global scale stood at 8% prevalence, and prevalence of undernutrition in sub-Saharan Africa was 23%. Undernutrition is usually not a direct cause of death, but undernutrition results in a child experiencing increased episodes of infections, and the severity of infection increases with each episode. Undernutrition further contributes to poor or delayed recovery^(1,2).

UNICEF reported that globally, in 2013, 161 million children were stunted. One-third of these 161 million children reside in Africa⁽²⁾. In 14% to 17% of child deaths globally, stunting was found to be an underlying cause of death⁽¹⁻³⁾.

According to the South African (SA) National HIV Prevalence, Incidence and Behaviour Survey of 2012, SA's national estimate of HIV prevalence is 12.2%. When compared to the same survey in 2008, 1.2 million more people were living with HIV in SA in 2012. The prevalence of HIV in the 0 to 14-year age group was 2.4%⁽⁵⁾. HIV prevalence was presented per age group in the 2012 survey as follows: 1.7% in the 0- to 4-year-old category and 2.7% in the 5- to 14-year-old category. Across provinces, the Western Cape had the lowest HIV prevalence in SA at 5.0% and KwaZulu-Natal had the highest HIV prevalence rate at 16.9%. HIV prevalence according to urban and rural location stood as follows: 10.1% HIV prevalence in urban formal settlements, 19.9% in urban informal settlements, 13.4% in rural

informal settlements and 10.4% in rural formal settlements⁽⁵⁾. The Medical Research Council of South Africa (MRC) 2012 prevention of mother-to-child transmission (PMTCT) evaluation estimated the prevalence of HIV infection within 4 to 8 weeks of birth in the Western Cape to be 0.4%⁽⁶⁾.

1.2 CAUSES AND CONSEQUENCES OF CHILDHOOD MALNUTRITION

The term malnutrition refers to both undernutrition and overnutrition. Undernutrition in children broadly refers to inadequate energy intake and low-weight-for-age and/or low-height-for-age, while overnutrition refers to excessive energy intake and overweight-for-age – both these result in ill health⁽⁷⁾.

This thesis focuses only on childhood undernutrition as a form of malnutrition⁽⁸⁾. The direct causes of undernutrition are poverty, lack of quality food, including low dietary diversity, poor feeding practices and care of infants and young children, micronutrient deficiencies and chronic or repeated episodes of infectious diseases. One cause of such a chronic state of infection and disease is HIV. Underlying these direct causes of undernutrition are low family income, poor environment and housing and lack of access to safe water and healthcare^(2,9,10).

The consequences of undernutrition can be short term and long term. These include poor growth, delayed mental development, increased prevalence of infectious diseases, poor performance at school, decreased earning potential and an increased susceptibility to chronic diseases later in life⁽¹⁰⁻¹³⁾. In addition, chronic undernutrition results in stunting⁽¹⁰⁾. Stunting is defined as a height-for-age Z score (HAZ) of equal or less than -2 standard deviations of the WHO child-growth standard median⁽¹⁴⁾. Stunting already starts in utero and continues during the early years. Stunting is an indicator for early-life chronic malnutrition and after two years of age, stunting is less reversible because the main growth and development occurs from 0-24 months. The exact causes of stunting remain unclear, however, macronutrients and micronutrients ante- and post-natally, infectious diseases and chronic inflammation are all contributors to poor growth. Perignon et al investigated stunting as a risk factor for lower cognitive performance. In their study they found that children above ten years of age had higher prevalence of stunting than children below ten years of age. They reported possible reasons for this, and suggested that the stunting in the region had improved in the last decade and also suggested the possibility that growth faltering continues after five years of age⁽⁴⁾. Figure 1.1 depicts a sequence of health events, starting at maternal undernutrition resulting in delivery of a low birth-weight infant. Inadequate macronutrient and micronutrient intake and prolonged infections due to poor immunity from

the poor nutritional status result in continued poor growth. The low birth-weight infant can become a stunted child and adolescent if nutrition and care do not improve after birth and in early life. In this way the consequences of stunting can extend into adulthood and can be transmitted from one generation to another⁽⁵⁾.

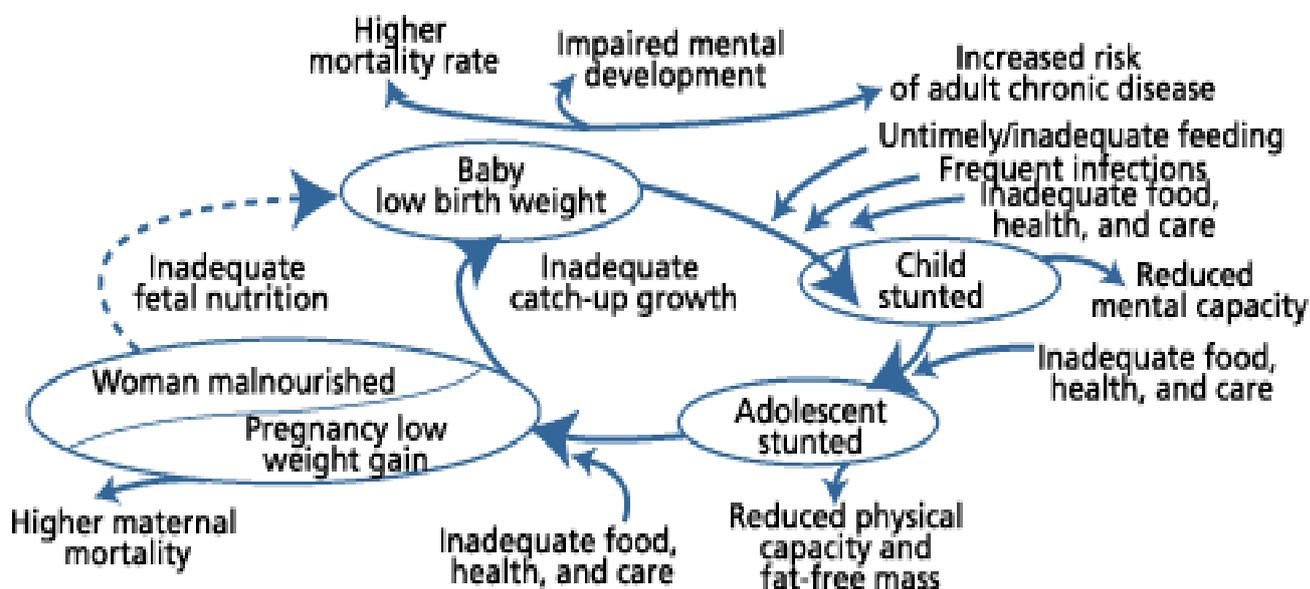


Figure 1.1: Poor nutrition throughout the lifecycle⁽⁶⁾

1.3 IMPACT OF URBANISATION ON CHILDHOOD UNDERNUTRITION AND HIV STATUS

There is an increasing number of children found to be growing up in cities and towns rather than in rural or farmland areas⁽⁷⁾. This is mainly due to urban cities and towns attracting individuals through better employment prospects and investments, and often being associated with economic development. Therefore children potentially have better opportunities to access health, protection, education and sanitation. Developing countries are also experiencing rapid population growth – two continents affected by this are Asia and Africa⁽⁷⁾. However, urban advancement has not been equal in all urban areas and many children are found to be residing in marginalized urban informal settings, referred to as slum areas. These slum areas can be squatter settlements and informal dwellings, and they are overcrowded and lack basic necessities⁽⁷⁾. The United Nations Human Settlements Programme defines a slum household as one that lacks one or more of the following:

- Access to improved water
- Access to improved sanitation
- Security of tenure

- Durability of housing
- Sufficient living area

The inequality that exists between the urban formal and urban informal settlement areas is due to the rapid population growth, which results in the need for increased healthcare, housing, employment and electricity. To sustain this growth, authorities attempt to increase the infrastructure of a city beyond its actual capabilities. Urban city residents in poorer areas are in close proximity to essential services such as hospitals and schools. However, many of these individuals choose not to access these services because of a lack of entitlement and empowerment or a perception that these services do not fall in their social or economic domain. This results in essential services becoming unavailable to children and families⁽⁷⁾.

As the poor and undernourished are increasing in urban areas, a significant shift is happening in the transfer of poverty and undernutrition from rural communities to urban communities⁽⁸⁾. In sub-Saharan Africa evidence was found of differences in child nutrition between the rich urban and poor urban communities. These differences were greater than the differences between urban and rural areas. In 2004, evidence showed that the energy-deficit proportion of the urban informal population was approximately 40% in almost all of the ten sub-Saharan countries studied⁽⁹⁾.

Lack of safe drinking water and sanitation, as well as poor hygiene, increase the risks of illness, undernutrition and death in poor urban families and result in many households being caught up in the so-called poverty trap⁽⁷⁾.

The total South African population grew from 40.6 million in 1996 to 51.8 million in 2011. Despite efforts to accelerate the provision of basic social services, in 2012 8.8% of the population had no access to piped water, 43% had no flushing toilets and 16% did not have electricity in their homes⁽¹⁰⁾. The Western Cape population grew by 29.3% between 2001 and 2011. This resulted in an increase of 37.5% in the number of households in the province. This growth placed much strain on the cities' infrastructure and service delivery programmes and resulted in 13% of households having no access to piped water and 12% having no flushing toilets. In addition, those who are able to work struggle to find employment – a situation that has resulted in many households in the Western Cape being struck by, among other things, food insecurity⁽¹⁰⁻¹²⁾.

“Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life”⁽¹³⁾. If these conditions are not met, then food insecurity exists⁽¹⁴⁾.

In the National Health and Nutrition Examination Survey (SANHANES) of 2014, 45.6% of the South African population were found to be food secure, 28% were found to be at risk of hunger and 26% of the population had experienced hunger in the previous month. The largest proportion of the population to experience food insecurity was in the urban informal areas (32%) and the rural formal areas (37%). The rate of hunger by province showed that the Gauteng and Western Cape populations experienced low rates of hunger when compared to the Eastern Cape and Limpopo populations, which had hunger rates above 30%. When aggregated by race group, the black African population had the highest rate of food insecurity (30%), followed by the mixed ancestry population (13%). While this data indicate that, at the national level, the South African population has a sufficient supply of food, it does not necessarily mean that there is sufficient food at the household level⁽¹⁵⁾.

Frayne et al found in 2010 that 80% of households in the Western Cape were either severely or moderately food insecure. They also found that urban food insecurity in Cape Town was due to the dependence on the cash economy. This meant that as food costs increase, food insecurity would result in the household, since urban dwellers do not farm or grow their own produce. The consequence is that urban dwellers are becoming more food insecure when compared to their rural counterparts, who in general grow their own produce⁽¹⁶⁾. Poor dietary diversity was also reported in a study by Battersby et al, where it was found that the most commonly consumed foods were mainly energy dense, such as fats, sugar or honey, but were low in other nutrients⁽⁹⁾. Household food insecurity results in limited choices of food, reduction in the number of meals per day and reduction of the portions of food at each meal⁽¹²⁾. In addition, developing countries are known for poor feeding practices. Much of these poor practices, particularly infant and young child feeding, are related to poor educational status of mothers and lack of nutritional knowledge of caregivers⁽¹²⁾.

In the developing world, women tend to be the main caregiver, the main purchaser of food and the person that prepares the meals for the family⁽¹²⁾. Where the mother is HIV positive and prone to illness due to HIV, it is found to contribute to child undernutrition. There is evidence that timely initiation of antiretroviral treatment for HIV-infected mothers can reduce the under-five mortality rate to a level similar to the rate for children of HIV-negative mothers⁽¹⁴⁾.

HIV, undernutrition and food insecurity has complex effects on the household and the individual. The three different dimensions of food security impacted by HIV infection is food utilization, food access and food availability. All three dimensions will worsen the food insecurity within a community.

At a household level, lack of food can result in risky coping behaviour such as selling household items and prostitution for food. This in turn places a further constraint on the economic situation of the home and increases the exposure to HIV⁽¹⁷⁾. If the cycle continues, it results in faster progression to AIDS.

People living with HIV infection face a multitude of difficulties, including the social and clinical impacts of HIV. They struggle with poor productivity since they are often unable to remain in employment as their health deteriorates. Loss of income and food production capacity results in reduced food intake. Adverse effects of medication can also negatively impact the employment capacity of the HIV-positive individual. This further results in difficult choices having to be made, for example choosing between essentials such as food, health care and schooling^(12,17,18).

Whether the parent or the child is infected with HIV/AIDS, the impacts are felt by the entire household. Children infected with HIV have nutritional requirements 50% to 100% above the normal requirements. If they are started on antiretroviral therapy (ART), their nutritional requirements are further increased as ART increases the resting energy expenditure of children. For some, the introduction of ART does improve nutritional status, but for others poor nutritional status persists despite being on treatment because of the increased energy requirements coupled with reduction in appetite while on treatment⁽¹⁹⁾. Undernutrition increases the susceptibility of hepatic toxicity to nevirapine. Ingestion of food and a well-functioning absorptive capacity of the gut is required to ensure the appropriate absorption of ART drugs, to ensure the effectiveness of the drugs and to achieve the full benefits of ART. In addition, HIV infection becomes a chronic infection resulting in a constant catabolic state which requires additional energy in the form of proteins, fat and carbohydrates. These macronutrients are known to be broken down rapidly in catabolic states and thus become depleted quickly^(3,20). Nutritional needs will differ according to the stage of HIV infection. If the child is underweight and has recently started on ART, they will be identified as being in the recovery stages and this will require 50% to 100% additional energy intake. However, once the child is stable on ART and no longer underweight, the additional energy requirements will be 10% extra due to the HIV infection. During opportunistic infections the HIV-positive child will require additional energy⁽²¹⁾.

Through the effects of food insecurity and the HIV status of the mother, all children in HIV households are at a greater risk of malnutrition than children living in an HIV-free household, where neither parent has HIV/AIDS⁽¹⁴⁾.

1.4 CO-EXISTENCE OF HIV AND CHILDHOOD UNDERNUTRITION

Many research studies have reported on the overlapping, the interaction or the co-existence of HIV and undernutrition^(3,7,14). It is often difficult to differentiate between or identify the root causes, and its consequences, of the undernutrition in HIV-positive children, as it may be due to food insecurity or due to the inflammation caused by the HIV virus⁽¹⁴⁾. Undernutrition, through its adverse effect on the immune system, can worsen HIV and result in death due to its acceleration of progression to AIDS. This happens because the HIV infection increases the individual's susceptibility to opportunistic infections. At the same time, the HIV infection also worsens undernutrition in different ways, including increased nutritional needs, altered metabolism, malabsorption and low food intake.

Trehan and colleagues reported that of the 137 million children under five years of age residing in sub-Saharan Africa, 12.3 million are underweight and 2.3 million are infected with HIV. The authors postulated that there is likely an overlap between these two groups⁽²²⁾. A meta-analysis reported in an article by Rose and colleagues suggested that 29.2% of children in sub-Saharan Africa who were underweight were also HIV positive. Rose and colleagues concluded that mortality is three times greater in underweight children who were HIV positive than in their HIV-negative counterparts⁽¹⁴⁾. Twenty nine percent (29%) of children admitted for severe malnutrition to 17 medical facilities in Sub-Saharan Africa were also HIV-infected. Referral hospitals in urban communities had a higher HIV prevalence rate the HIV-infected children with severe malnutrition compared to children without malnutrition. These children were found not to be on ART and malnutrition was the consequent AIDS-defining illness⁽²²⁾.

In a study comparing weight of HIV-uninfected children to HIV-infected children, it was found that if HIV-infected children were undernourished, they tended to be more stunted and underweight than the HIV-uninfected children. The study also reported that being underweight was associated with the presence of other conditions, including candidiasis and cutaneous infections. These conditions resulted in further increases in energy requirements⁽²²⁾. In another study, height and weight was compared for a cohort of 184 HIV-infected children and a cohort of 1 403 HIV-uninfected children from birth through to ten years of age. Within the ten years, weight and height was measured at 24 time points. Analysis of the results showed that, although there was no significant difference between the groups at birth in these indices, significant differences became evident as the children advanced in age. The study found that the uninfected children were significantly taller and heavier from an early age. This was followed up again at 3-4 years, when it was reported that the uninfected children grew an estimated 10.7% in height and 10.8% in weight faster

than the HIV-infected children. By ten years of age the children in the uninfected group were 7kg heavier and 7.5cm taller than the infected children⁽¹⁴⁾.

1.5 UNDERWEIGHT IMPACTS ON THE IMMUNE SYSTEM

The immune system's major function is to protect its host from infectious agents found in the surrounding environment. There are two main arms of the immune system, namely the innate immune system, which is the first line of defence, and the acquired immune system, which is responsible for the specific recognition of molecules. When a foreign body is encountered by the immune system, the innate response with its inflammatory component will be activated. The inflammatory response will start by releasing cytokines via macrophages and monocytes. They will then stimulate the liver to produce acute-phase proteins and act on the brain to reduce appetite. The cytokines will trigger activation of the acquired immune response by stimulating the T lymphocytes. T lymphocyte activation will result in proliferation of B and T lymphocytes to promote antibody production. This integration of responses will contribute to removal of the antigen and provide an immunological memory⁽²³⁾.

The strength, the magnitude and type of immune response developed by an individual is dependent on a variety of factors. Factors include the immediate environment, infection, age, genetics, nutrient status and early life events⁽²³⁾.

Undernutrition negatively affects the immune system. Micronutrient and macronutrient deficiencies weaken the immune system via impairment to the thymus and lymphoid tissue and other immune cascade reactions. Poor immunity results in an increased risk of infection and disease progression⁽¹⁹⁾. These deficiencies increase the susceptibility of infections in the gastrointestinal tract (GIT). This impairs the absorption of nutrients which further worsens the immune function⁽²⁴⁾.

Nutritional status is an important determinant of the susceptibility to, as well as the course and outcome of an infection. Protection from micro-organisms requires an intact skin surface, and intact lining of the mucosal surfaces, including, naso-oesophageal, gastrointestinal and genito-urinary tract. For protection against infection to be maintained, the cells lining these barriers are continuously differentiating and replicating. The immune response to an infection entails an increase in cell differentiation and replication, production of immunoglobulins and acute-phase proteins, and the production of peptide and lipid-mediators. This response therefore requires an appropriate supply of nutrients. However, in the case of an undernourished individual, the immune response will be negatively affected

because these individuals do not have an optimal supply of nutrients and thus immune cells functions will be impaired⁽¹⁴⁾.

The combined effect of undernutrition and HIV on the immune system will affect and ultimately worsen the prognosis of the individual, because the two conditions operate in a self-reinforcing cycle which results in tissue depletion, and lowered resistance to diseases. Table 1.1 provides a list of immunological biomarkers and the effects of malnutrition and HIV on these biomarkers⁽²⁰⁾.

Table 1.1: Immunological biomarkers affected by HIV infection, malnutrition and nutrient deficiency⁽²⁰⁾

Immunological Biomarkers	Effect of HIV Infection	Effect of Malnutrition	Nutrient Deficiency
<i>T lymphocytes</i>	Decreased	Decreased	Protein Energy Malnutrition (PEM)
<i>B lymphocytes</i>	Polyclonal activation	Generally maintained	-
<i>Cell-mediated immunity</i>	Compromised	Compromised	PEM, essential amino acids
<i>Immunoglobulins levels</i>	Increased Immunoglobulin A (IgA)	Reduced IgA	PEM, amino acids, vitamin B complex
<i>Secretory IgA</i>	Increased	Decreased	PEM
<i>C-Reactive protein</i>	Increased, marker of HIV disease progression	Increased	PEM

The gastrointestinal tract (GIT) is one of the earliest organs to be affected by HIV infection. HIV infection is associated with nutritional deficiencies due to its effect on nutrient absorption⁽¹⁹⁾. The result is impaired intestinal cells that cause flattening of the villi and decreased D-xylose absorption, which leads to poor absorption of nutrients, in particular carbohydrates and fat. Alteration of epithelial barrier function leads to bacterial translocation and consequently to infections and episodes of diarrhoea, which further worsens the nutritional status of the individual^(3,14,20,25). Macronutrients are required for the regeneration and maintenance of the immune response. T-cell metabolism requires glucose, amino acids and fatty acids. A deficiency in glucose can result in negative impacts on proliferation and cytokine expression. Deficiencies in amino acids reduce immune activation. Moreover, evidence reported by Kau et al indicated that deficiencies of vitamin A, D, and E and zinc due to undernutrition can adversely impact the immune function⁽²⁴⁾. Immune impairment leads to further malnutrition and stunting. The combination of undernutrition and the impaired immune system would then contribute to rapid progression to AIDS⁽²⁰⁾.

1.6 HIV, INFLAMMATION AND INFLAMMATORY MARKERS

The HIV virions found on the surface of the dendritic cells in the germinal centre of the lymphoid organs, where they are periodically released, result in a steady chronic state of infection and a chronic inflammatory response^(25,26). Inflammatory response refers to the complex set of reactions following infection. The inflammatory response is the homeostatic process that takes place to normalise the environment in the event of an infection. This response is activated to prevent further tissue impairment or to attempt to remove the infection. The changes that occur metabolically are taxing and can potentially cause irreversible impairment. The inflammatory response can be potentially destructive, therefore the inflammatory response should be well controlled and not last a long period of time⁽²⁷⁾. The set of reactions that start directly after the infection is known as the acute-phase response⁽²⁷⁾. Cytokines are essential for co-ordinating the inflammatory response by either playing a pro-inflammatory role to initiate defence or an anti-inflammatory role to down-regulate the inflammatory process and avoid exacerbated reactions^(3,20).

C-reactive protein (CRP) is used as a biomarker to measure the inflammatory state of the body. CRP is an acute-phase protein produced by the hepatocytes and other cells in response to cytokines, such as Interleukin 6 (IL-6). High levels of circulating CRP are caused by infection and chronic disease⁽²⁵⁾. The introduction of ART has been successful in suppressing HIV replication and significantly reducing opportunistic disease. However, many studies found that individuals successfully managed on ART are still at high risk for mortality due to other conditions such as cardiovascular disease with chronic inflammation being the common denominator^(27,28). Two well-known studies, the SMART and FIRST studies, both large randomized clinical trials, identifying inflammatory markers in HIV, found that increased levels of circulating CRP was a predictor of mortality and opportunistic infections in individuals who were HIV-infected and receiving ART. They however reported that there was uncertainty as to whether the increased CRP levels caused the mortality and incidence of opportunistic infections or whether it was just a marker of a global inflammatory state. They concluded that ART does not appear to reduce CRP significantly⁽²⁵⁾.

Borato et al also found CRP levels to be increased in HIV-infected individuals on ART. They concluded this effect to be due to the ongoing inflammation despite an undetectable virus level⁽²⁶⁾.

Pendergast and colleagues measured low-grade inflammation associated with stunting in 202 HIV-unexposed Zimbabwean infants. The 202 infants were sampled as follows: 101 non-stunted and 101 stunted infants at 18 months. They measured biomarkers of inflammation, one of which was CRP. They found that from six weeks of age through to one

year, the levels of CRP in the stunted group were consistently higher when compared to the control group⁽¹⁾.

Calprotectin, a calcium- and zinc-binding protein, which is released during inflammation process by neutrophils, resulting from mucosal damage, is used as a non-specific marker for gastrointestinal inflammation^(25,29). Elevated levels of calprotectin are found with infectious and inflammatory diseases⁽³⁰⁾. Faecal calprotectin is a useful marker of inflammation in paediatric diseases. It is a non-invasive test that is associated with the presence of mucosal inflammation, and together with C-reactive protein and other laboratory indices is effective in identifying paediatric colorectal inflammation⁽³⁰⁾.

Hestvik et al found that calprotectin can be used as an inexpensive test to investigate gut inflammation in children living in low-income countries. Studies have also reported faecal calprotectin concentrations to be elevated in both children and adults with inflammatory bowel disease^(29,31). HIV-positive Ugandan children above four years of age, on treatment, were found to have faecal calprotectin levels above the reference range⁽³⁰⁾.

Children under the age of two years have not yet a fully mature immune system. Children have higher T- and B-lymphocyte counts than adults, but these cells are immature and it takes the time from birth to about ten years for an optimal cell repertoire to be developed. During this period of development, children are therefore prone to infections⁽³²⁾. The B-lymphocyte cells form a component of the acquired immune system, which is responsible for developing a specific protective immune function by developing a memory to previous infections and therefore increasing protection for the future⁽³²⁾. The gastrointestinal tract is exposed to large amounts of bacteria after birth⁽³³⁾. The GIT is highly adapted to the presence of these bacteria and their immune-stimulatory effects⁽³³⁾. Immunoglobulins are found in abundance on the mucosal surfaces and provide a first line of defence⁽³³⁾. Experiments in animals found that animals kept in a germ-free state had a decreased number of immunoglobulin A(IgA)-producing cells when compared to animals containing intestinal bacteria which had a higher count of IgA-producing cells⁽³³⁾. IgA presence is therefore determined by the presence of intestinal bacteria. Immunoglobulins are proteins that attach to foreign substances, for example bacteria, and assist in destroying them. During an infection, IgA levels increase in an attempt to protect and bring about homeostatic control⁽³²⁾. The lack of IgA indicates a reduced activity of the intestinal immune system. An IgA deficiency is associated with an increase in upper respiratory and gastrointestinal infections⁽³²⁾.

There is evidence that IgA response is required to protect the GIT against intestinal pathogens such as rotavirus. Further evidence also shows that IgA response not only controls commensal bacteria, but can also neutralize microbial products with pro-inflammatory activity⁽³⁴⁾. Cerrutti et al found that if a dysregulation of antibody response occurs, commensal bacteria are not controlled and this results in an excessive innate immune response. This could aggravate intestinal inflammation and trigger inflammatory disorders such as celiac disease, IgA nephropathy and Crohn's disease⁽³³⁾.

The available evidence, therefore, indicates that the severity of the combined effect of HIV and malnutrition ultimately weakens the individual's immunity. Yolken et al found that 30% to 60% of asymptomatic HIV-infected children mal-absorb carbohydrates, 30% mal-absorb fat and 32% mal-absorb proteins⁽³⁵⁾. However, merely feeding these children is not the simple answer. The Food and Agricultural Organization (FAO) stated that countries need evidence-based nutrition interventions as part of their national HIV care and treatment programmes and that these interventions should include routine assessment of dietary intake, nutritional supplementation and monitoring of nutritional status, via measurements of height and weight, for individuals infected with HIV⁽³⁶⁾.

1.7 SOUTH AFRICAN GUIDELINES FOR THE MANAGEMENT OF HIV-POSITIVE CHILDREN (2010)

HIV disease progresses quicker in children than in adults and opportunistic infections occur sooner due to children's immature immune system. For this reason, the SA guidelines stipulate that children should be managed by routine follow-up visits for growth monitoring and promotion, as well as nutritional support⁽³⁷⁾.

The National Consolidated Guidelines for PMTCT state that HIV positive children who are classified as severely malnourished should be treated as any other Severe Acute Malnutrition (SAM) child. This document adds that symptomatic HIV children require an additional 30% energy to their normal intake, however, if this child is symptomatic and SAM, then an additional 100% energy is required for dietary intake. This is often impossible to achieve through intake, it is therefore recommended that these children are placed on the Nutritional supplementation programme within their province based at their primary healthcare clinic⁽³⁸⁾. The South African National guidelines 2015 for the management of children with acute malnutrition indicate that the severity of malnutrition can be classified and treated accordingly⁽³⁹⁾. For the relevance of this thesis, the guidelines for the management of moderate acute malnutrition (MAM) at outpatient supplementation programme will be briefly discussed. Children presenting with MAM can be managed by two options:

- Food based approach through enriching home diet. This involves dietary counselling and education to the caregiver on how to best enrich the child's dietary intake.
- Supplementation through ready-to-use supplementary feeds. This option will be used in the situation where the child was non-responsive to option 1 or the child resides in a food insecure home.

Irrespective of which option is selected, the child will be followed up initially every two weeks to assess weight gain. At these visits weight gain will be assessed, adherence to supplementary feeding regimen and medical illness will be assessed. These visits will include ongoing health education and nutrition counselling⁽³⁹⁾.

In the Western Cape this programme is referred to as the Nutrition Therapeutic Programme (NTP) and it provides a monthly supply of a maize-based instant porridge and lactose-free energy drink. However, inaccessibility of a clinic from which to collect the supplements, food insecurity within the household and maternal health often result in the child not receiving the full benefits of nutritional support⁽⁴⁰⁾.

1.8 NUTRITIONAL SUPPLEMENTATION AND HIV

Optimal nutrition has many benefits, including improving an individual's immune response, reducing episodes of complications associated with HIV infection, delaying progression to HIV infection, improving quality of life and potentially reducing mortality associated with disease⁽¹⁸⁾.

Figure 1.2 shows the potential benefits of nutritional supplementation in the context of HIV. These benefits include a strengthened immune system, reduced vulnerability to infections, a good nutritional status and nutritional needs being met. These factors can ultimately ensure that underweight individuals can regain sufficient weight which will result in their immune system being better equipped to fight the HIV infection and any other opportunistic infections which lead to slower progression to AIDS.

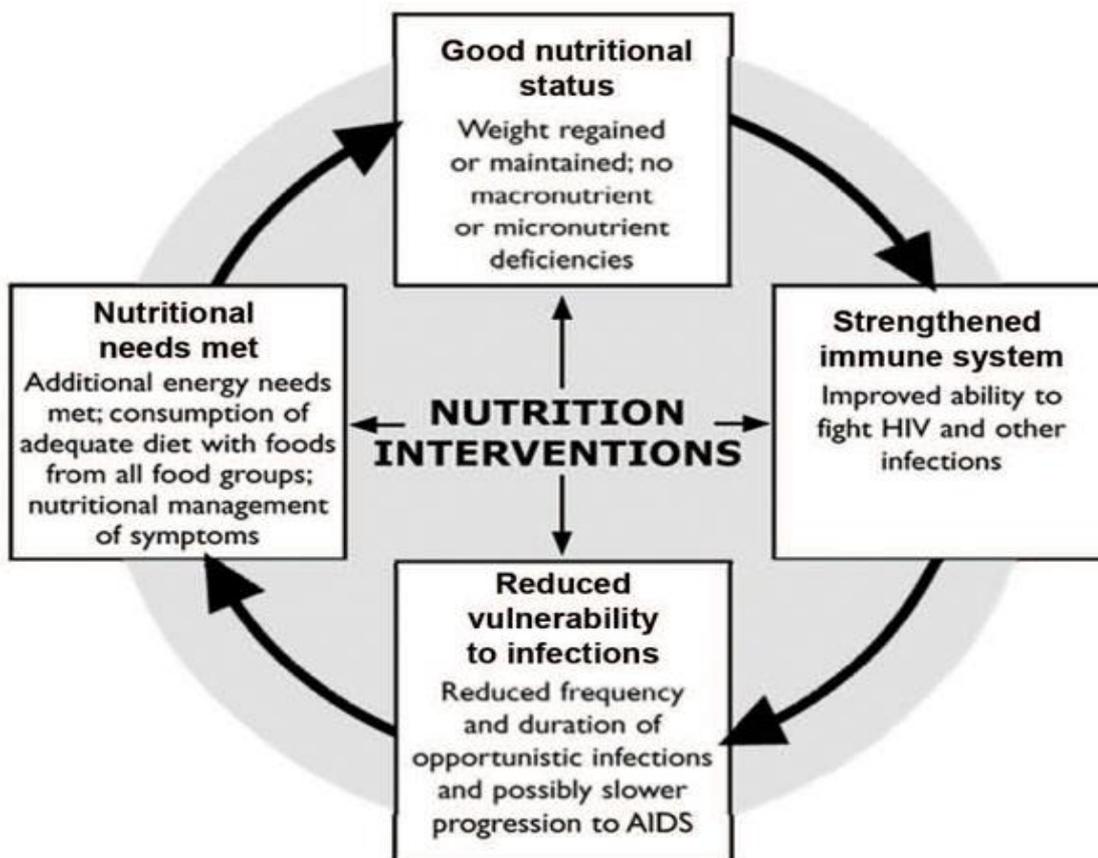


Figure 1.2: Potential benefits of optimal nutrition in the context of HIV/AIDS⁽⁴¹⁾

Providing dietary advice to improve nutritional status is not always possible due to unavailability of qualified staff in nutritional support/care or to inaccessibility for patients or to poor compliance. Weight loss may therefore continue even after advice is provided.

Supplementary feeding (SF) is defined as providing nutrition portions to specific individuals that need to supplement the energy and nutrients missing from their dietary intake. SF can be provided in two ways, these include, blanket supplementary feeding (BSF) and targeted supplementary feeding (TSF)⁽⁴²⁾.

BSF is provided to all individuals in an at-risk group irrespective of nutritional status, in comparison, TSF is provided to an individual who has a poor nutritional status, such as malnourished individuals or HIV positive individuals⁽⁴²⁾. A large percentage of the literature available is based on TSF.

Some studies have supplemented HIV-infected and underweight children and adults with macronutrients, while other studies have used single micronutrients with the aim to improve the nutritional status of these individuals. Food-insecure communities may not fare well with nutritional supplementation, as often the supplementation is utilised by the household as a

whole and not only by the individual, resulting in inadequate nutritional quantities that do not meet the needs of the HIV-infected or malnourished individual⁽³⁾.

Figure 1.3 portrays the factors that impact on food supplementation interventions for underweight and HIV-disease outcome. These factors include the characteristic of the food supplement, the characteristics of the individual, treatment adherence and education provided to an individual receiving supplementation with an assumed normal dietary intake.

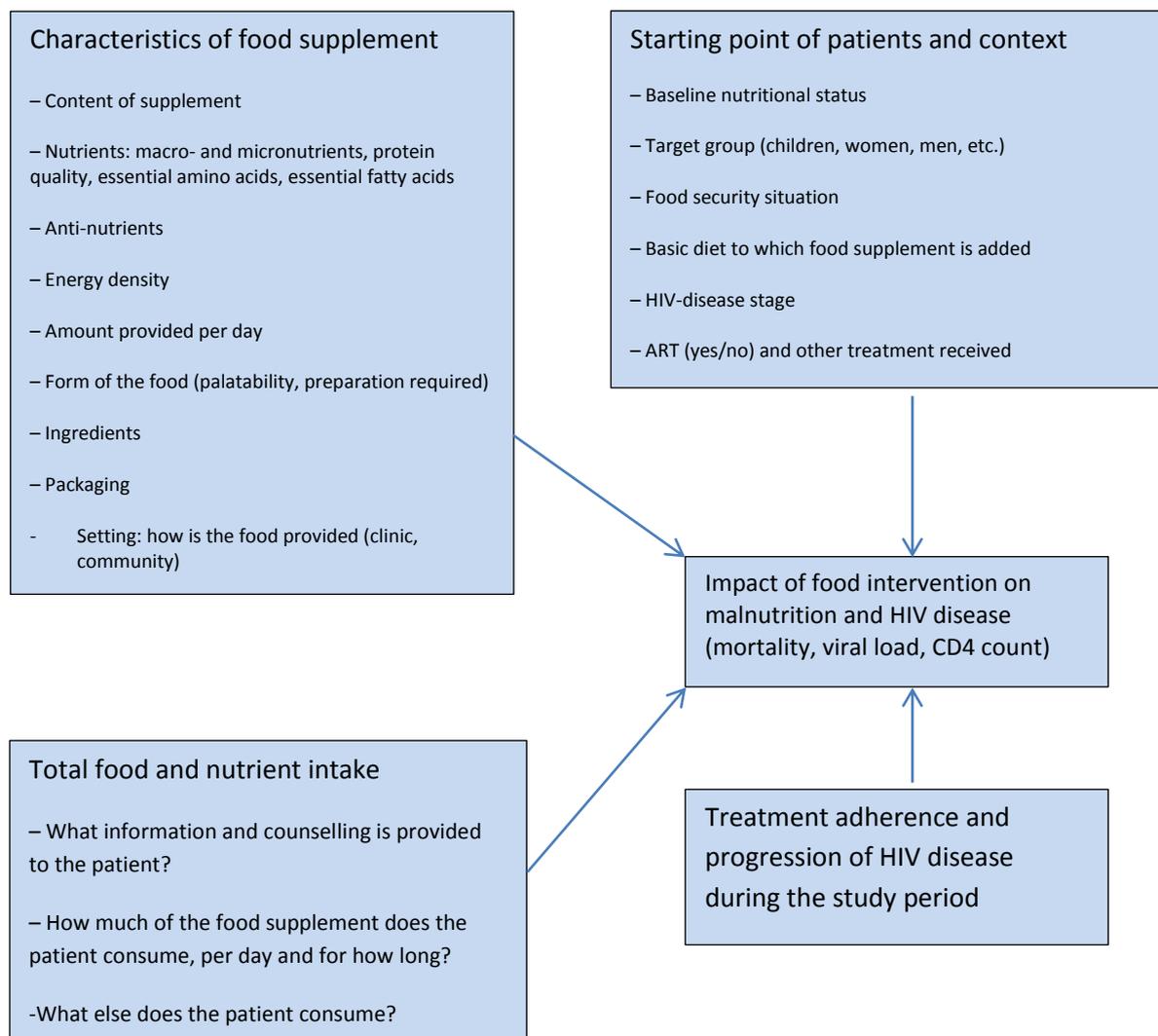


Figure 1.3: Challenges faced when introducing a nutritional supplement to underweight and HIV-infected individuals⁽³⁾

1.8.1 Nutritional supplementation: Macronutrients

Mahlungulu et al carried out a systematic review which considered the effectiveness of macronutrient interventions compared to no nutritional supplements provided (only dietary advice from a dietician) or a placebo (a supplement with low energy content) in the

management of adults and children infected with HIV. The review was based on eight clinical trials performed in high-income countries. They found limited evidence that macronutrient supplementation increased protein and energy intake. They also concluded that there was no evidence suggesting that such supplementation resulted in reductions in disease progression or HIV-related complications⁽¹⁸⁾.

1.8.1.1 Paediatric-specific studies

The paediatric studies available on the effects of supplementation were reported in a review by Sztam in 2010, where all the evidence available for macronutrient studies in underweight HIV-positive children was considered. All the reviewed studies focused on patient outcomes, namely during or after inpatient nutritional rehabilitation and children with severe malnutrition (SAM) and HIV infection. These studies showed no difference in mortality between the HIV-infected children with SAM attending an outpatient clinic and those attending a rehabilitation unit. Fourteen studies investigated inpatient outcomes of HIV-infected children provided with supplementation. In these studies, the study design did not have a comparative group nor did it utilise uninfected children as controls. Six studies investigated macronutrient supplementation on outpatients after a period of nutritional rehabilitation. None of these studies considered children on ART or children attending an outpatient unit where mild to moderate malnutrition would be found. Only three studies included HIV-infected comparative groups, randomized to receive different macronutrient supplements within an inpatient setting. There have been no studies on children who were HIV-infected, receiving supplementation and followed-up on in care. One of the reasons alluded to is the fact that SAM is often the most important or pressing health issue to attend to, especially in developing countries. The available data on ART are scant and the effects of nutritional supplementation and mild to moderate malnutrition with HIV. Where mild to moderate malnutrition was investigated, it was linked to SAM recovery/rehabilitation. No study considered ongoing child outpatients in HIV care and treatment programmes. The authors concluded that there is a need for supplementation studies prioritised for specific groups of children and adults who are HIV positive and on ART. Other important studies documented a need for interventions on HIV-positive children with mild to moderate malnutrition in an outpatient environment. This will allow for conclusive outcomes on whether nutritional supplementation is effective in HIV-infected children with mild to moderate malnutrition⁽⁴³⁾.

1.8.1.2 Adult-specific studies

Studies investigating macronutrient supplementation for adults are very limited. Indeed, there are limited published data reporting on effects of supplementation on CD4 count and

viral load. Two studies reported an improvement in body mass index (BMI) status and one study found improvement to adherence of ART^(18,43).

1.8.2 Nutritional supplementation: Micronutrients

The acute-phase response has serious nutritional implications. Ongoing inflammation can result in infection-induced loss of weight related to increased nutrient requirements and inadequate energy and nutrient intake due to poor appetite and/or absorptive capacity of the GIT. Micronutrient deficiencies result in metabolic alterations that include whole-protein turnover, increased urinary nitrogen loss and skeletal muscle breakdown which is paramount in the synthesis of immune regulators, such as proliferation of neutrophils, lymphocytes, fibroblasts, synthesis of immunoglobulins and acute-phase proteins⁽²²⁾, attendant morbidity⁽⁴³⁾ and organ-specific manifestations⁽²³⁾.

1.8.2.1 Paediatric-specific studies

Allen et al reported results from a meta-analysis of randomized controlled trials (RCTs) investigating the provision of multiple micronutrients (combination of vitamins and minerals) rather than two or less micronutrients to HIV-negative children and adults who were micronutrient deficient. Most of the studies included in the meta-analysis involved infants and pre-schoolers, and five studies included school-going children. In all these studies, the multiple micronutrient supplementations (MMS) had positive effects on height of the children, compared to the control groups⁽⁵⁾. One of the studies reported in the meta-analysis carried out in Vietnam investigated MMS on stunted children. This was the only study that considered stunting as an outcome. It found that if at baseline the child was stunted, they had a significant increase in linear growth due to receiving either daily or weekly MMS compared to the placebo. At the time of publishing the study there was no knowledge of studies done on children who were HIV positive, so they could not review HIV-positive children receiving multiple micronutrients⁽⁵⁾.

1.8.2.2 Adult-specific studies

Allen et al did consider RCTs of MMS for HIV-positive adults. The results were that supplementation with multiple micronutrients reduced mortality and in some cases morbidity in adults with HIV. However, more studies are required to properly investigate MMS in adults. Two RCTs examined the benefits of MMS in patients on Highly Active Antiretroviral Therapy (HAART)⁽⁵⁾. In the first RCT, in Poland, the investigators found that supplementation with multiple micronutrients for six months improved immune function, but caused no change in CD4 count when compared with a placebo. In the USA, the second RCT reported no negative effects when giving MMS in conjunction with HAART on fasting blood glucose, lipids or insulin. This RCT also found that after 12 weeks of supplementation, the group

receiving the MMS had higher CD4 counts than those receiving the placebo. Allen and colleagues did conclude that these RCTs were of a small scale and their outcomes were few⁽⁵⁾.

1.8.3 Nutrition supplementation: Probiotics & prebiotics

The immune system of the GIT can be influenced by dietary components and composition, as well as metabolic activity of commensal bacteria. Prebiotics and probiotics are hypothesised to modulate the microbial composition and the response of the GIT immune system of an individual⁽⁴⁴⁾.

Probiotics are defined by the WHO as “live micro-organisms that can provide benefits to human health when administered in adequate amounts”⁽²¹⁾. Vieira and colleagues defined prebiotics as a food ingredient made up of oligosaccharides, which are not digestible by an individual and have beneficial effects on the individual’s health via action on growth and activity of gut microbiota⁽⁴⁴⁾.

Probiotics and prebiotics modulate the innate and adaptive immune response.

Probiotics have anti-pathogen properties. They may modulate immune functions resulting in an increase in the cytotoxic capabilities of natural killer cells, as well as an increase in the phagocytosis capabilities of macrophages. Both the natural killer cells and the macrophages are innate immune cells. Probiotics can therefore improve the mechanism of pathogen destruction, which reflects an improvement of the functioning of the immune system⁽⁴⁵⁾. Probiotics are thought to modulate the adaptive immune response by stimulating B cells to produce IgA, which binds antigens and limits their access into the epithelium. Probiotics also act on cells of the immune system to modulate inflammatory reaction. The evidence, however, strongly associates this to specific strains of probiotics^(44,45).

Prebiotics can favourably promote the growth of selected bacterial species, including bifidobacteria, which is known to be indicator of healthy gut condition. Fermentation of carbohydrates results in the generation of short-chain fatty acids (SCFA). SCFA are prebiotics and form a source of potential fuel for the epithelial cells of the GIT⁽⁴⁶⁾. Prebiotics increase intestinal barrier function by exerting an antimicrobial effect because they adhere to binding sites of bacteria on the enterocyte surface and can therefore block the adhesion of pathogenic bacteria to the intestinal epithelial cells⁽⁴⁷⁾. Prebiotics also reduce GIT infections by reducing the number of faecal pathogens in the GIT and increasing production of IgA, which has homeostatic effects in the GIT⁽⁴⁶⁾. Prebiotics have the potential to increase the ability of cells of the immune system to carry out transcriptional activities⁽⁴⁵⁾. It is postulated

that such immune modulatory properties may then change the interactions between the host and microbes⁽⁴⁶⁾. HIV infection has a negative impact on the physiological interaction between the commensal microbiota and the immune system. CD4 cells are depleted, there are reduced dendritic cells and the composition of the intestinal epithelium is changed. The net result is increased susceptibility to GIT infections. HIV infection is also associated with immune activation and inflammation, which further fuels the replication of the virus⁽⁴⁶⁾. Probiotics and prebiotics therefore may have the potential to benefit the HIV-positive individual by improving their immune status through immune modulation.

In a review published by Cunningham-Rundles et al, current and emerging studies supported the idea of probiotic bacteria providing benefits to children who are infected before the development of normal gut flora and who are at risk of growth abnormalities and chronic inflammation⁽⁴⁸⁾. Van Niekerk et al found that prebiotics had a beneficial role in the decreased risk of developing necrotising enterocolitis (NEC) in HIV-exposed preterm infants. Van Niekerk et al also carried out a randomized clinical trial investigating the use of probiotics in the development of NEC in HIV-exposed versus HIV-unexposed low birth-weight infants. The results of this trial showed a significantly lower incidence of NEC in the group supplemented with probiotics than in the control group⁽⁴⁷⁾.

In a review by Hummelen et al, it was concluded that in recent years there has been initial evidence presented by studies on the use of pre- and probiotics in HIV disease and that these studies have shown that there are some benefits for intestinal homeostasis. Probiotics were used to reduce bacterial translocation in people from different population groups, with varying degrees of success as the outcome. In another RCT where 65 critically ill patients were given probiotics, patients showed reduced rates of infections, sepsis and mortality. However, the need remains for well-designed double-blind, randomized studies to provide specific evidence for various strains of probiotic interventions⁽⁴⁶⁾.

1.8.4 Nutritional supplementation: Immuno-modulators

Poly-unsaturated fatty acids (PUFA) have a role in the regulation of the inflammatory response. Increased intake of n-3 PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), increase the amount of fatty acids in inflammatory cell phospholipids, which indicates their involvement in determining the development and severity of the inflammatory response. This is because eicosanoids are generated from PUFA and they are one of the important mediators and regulators of inflammation and the immune response. DHA in cells produces less inflammatory eicosanoids, and this in turn results in less local and systemic inflammation^(49,50).

The beneficial effects of n-3 PUFA on immune markers, including T-lymphocyte proliferation, natural killer cell activity, and cytokine production by monocytes, have been investigated in healthy subjects based on dose-dependent effects. DHA in cells produces less inflammatory eicosanoids. This in turn results in less local and systemic inflammation. Animal studies have shown that this fatty acid has powerful immune-modulatory effects. In humans, one study that involved supplementing the cohort with fish oil supplements resulted in 80% reduction in pro-inflammatory cytokine production. In addition, there are studies that support fish intake or fish oil supplementation as a form of prevention of cardiovascular disease. Its effect was by means of reduction in blood pressure, platelet aggregation and pro-inflammatory eicosanoids. Further evidence is needed with regard to chronic inflammatory conditions^(49,50).

Studies on DHA show that it appears to impact on immune functions. However, more studies are needed on the impact DHA supplementation can provide to HIV-positive individuals⁽⁴⁹⁾.

1.9 MOTIVATION FOR THIS THESIS

There is a lack of evidence available on children who are HIV positive, managed on ART and receiving a nutritional supplement that contains enriched macronutrients, micronutrients and immune-modulators. This lack of evidence presents a problem in addressing the challenge of mild to severe malnutrition in the context of HIV. Evidence is also needed to examine the effects of an enriched supplement on immune markers and the impact of nutrients such as probiotics, prebiotics and DHA on HIV-positive children`s height, weight and inflammation.

This thesis formed part of a large randomized clinical trial. The primary objective of the clinical trial was to determine whether a complete nutrient supplement with added prebiotics, probiotics and DHA, as compared to the same supplement without these added nutrients, can lead to catching up in weight in HIV-positive children aged 24 to 72 months. The trial aimed at showing non-inferiority between the two supplements with regard to catching up in weight, thus further documenting the safety of such supplements in the HIV context. Therefore the design for a non-inferiority study would require groups randomized and large sample sizes to achieve sufficient power to draw conclusions.

The main report from the clinical trial showed that there were no major or significant differences between the two intervention groups with regard to the primary outcome, i.e. catching up in growth, thus further documenting the safety of using functional nutrients, including pre-probiotics, in such sensitive HIV-positive children populations. No superiority of the pre-probiotic-containing product was observed in primary outcomes probably because the trial was not powered enough for such efficacy endpoint.

Therefore, this thesis aimed at further exploring the impact of a complete supplement with high nutritional value, and enriched with functional nutrients, on growth and immune markers in a more global perspective. To do so, the whole cohort was assessed, irrespective of the supplement composition received by the children. In addition, this thesis explored whether the entire cohort, as well as children receiving either of the two supplements separately, showed associated changes in the prevalence of defined anthropometric indices (height, weight and derivatives thereof), and in defined immune and inflammatory markers for HIV-positive children within a child outpatient healthcare setting. The exploration of the children from the two separate groups allowed for the determination of bio-equivalency of the two supplements.

CHAPTER 2: METHODOLOGY

2.1 AIM

The aim of this study was:

To determine the effect of a nutritional supplement containing or not additional (prebiotics, probiotics and DHA) nutrients and given in addition to the daily food intake on growth (weight and height) and selected immune markers (C-reactive protein, faecal IgA and faecal calprotectin) in a cohort of HIV positive children aged 24-72 months.

2.2 OBJECTIVES

1. To determine the effect of the nutritional supplement given as described on the growth (height and weight) of a cohort of underweight HIV positive children aged 24-72 months.
2. To determine the effect of a complete nutritional supplement given as described on the levels of selected immune markers (C-reactive protein, faecal calprotectin and faecal IgA) of a cohort of underweight HIV positive children aged 24-72 months.
3. To explore more specifically how the added nutrients (prebiotics, probiotics and DHA) in the nutritional supplement influence the measured parameters on growth (height and weight) and selected immune markers (C-reactive protein, faecal IgA and faecal calprotectin) of a cohort of underweight HIV positive children aged 24-72 month to determine bio-equivalency of the two supplements.

It was deemed appropriate to provide background information on dietary intake of participants to enhance the context of the impact, if any, of the complete nutritional supplement on growth and selected immune markers. However, dietary intake was recorded and analysed for the clinical trial report but did not form an objective in this sub-study.

2.3 HYPOTHESIS

A complete nutritional supplement as described will improve the growth (height and weight) and level of selected immune markers (C-reactive protein, IgA and calprotectin) of a cohort of underweight HIV positive children aged 24-72 months, measured during the period baseline to visit 6 (seven months of supplementation).

A complete nutritional supplement with added nutrients will improve growth (height and weight) and level of selected immune markers (C-reactive protein, IgA and calprotectin) of a

cohort of underweight HIV positive children aged 24-72 months, measured during the period baseline to visit 6 (seven months of supplementation). Bio-equivalence will be established for both complete supplements.

2.4 Study setting

Cape Town is the second most densely populated city in South Africa and is the provincial capital of the Western Cape Province. Cape Town is a predominantly urban city which comprises of 78% of the residents living in formal dwellings and the remaining 22% living in slum areas referred to as informal dwellings. The population consists of 42% mixed ancestry, 39% Black African and 19% Caucasian people (Stats SA 2011). Figure 2.1 provides a geographical representation of Cape Town within the Western Cape Province.



Figure 2.1: Geographical representation of Cape Town City within the Western Cape Province (City of Cape Town 2004)

Cape Town is identified within the Department of Health structure as the City of Cape Town district which is divided into four health sub-districts, namely:

1. Northern and Tygerberg
2. Western and Southern
3. Mitchells Plain and Klipfontein
4. Eastern and Khayelitsha

Each sub-district has its own Primary Health care facilities which are referred to as Clinics and Community Health Centres, level 1 hospital services, health programmes such as HIV/TB, maternal health and child care and pharmacy services. The hospital services include secondary and tertiary facilities. Table 2.1 provides an overview of each secondary and tertiary hospital within a sub-structure.

Table 2.1: Cape Town City health district's health facilities

Sub-district	Secondary hospital	Tertiary hospital	Drainage Primary health centre
Northern and Tygerberg	Karl Bremer hospital	Tygerberg Academic Hospital	Clinics and Day hospitals
Western and Southern	New Somerset hospital	Groote Schuur Academic Hospital	Clinics and Day hospitals
Mitchells Plain and Klipfontein	GF Jooste hospital and Mitchells Plain hospital	Groote Schuur Academic Hospital	Clinics and Day hospitals
Eastern and Khayelitsha	Khayelitsha hospital	Tygerberg Academic Hospital, Groote Schuur Academic Hospital and Helderberg hospital	Clinics and Day hospitals

The provincial HIV/TB directorate's mandate is decentralised and operational in all of the four sub-structures and provides preventative, diagnostic, and treatment services. Anti-Retroviral clinics are located at both hospitals and clinics. Both adults and children are attended to at these facilities. The study site for this specific research project was located in the Northern and Tygerberg sub-District, based at the Tygerberg Academic Hospital.

2.5 STUDY DESIGN

On page 30 and 31 of the thesis, under the heading motivation for the study, the author has described that this thesis forms part of a sub-study within a larger clinical trial. The over-arching aim of the clinical trial was to assess non-inferiority between two complete nutritional supplements on catch-up weight. One of the supplements contained additional functional nutrients (probiotics, prebiotics and DHA). The sub-study however, does not compare two nutritional supplements and their effects on growth and selected immune markers in objectives 1 and 2, but rather looks globally at a cohort of underweight HIV positive children receiving additional nutritional support to their daily intake, and analyses the effect of the supplements on growth and selected immune markers. In objective 3, a sub-group analysis is carried out to determine bio-equivalence to the standard treatment, therefore the cohort is divided into those receiving supplement A and those receiving supplement B. The study design of the over-arching clinical trial was to test non-inferiority. The sub-study data for this thesis are those of the clinical trial and therefore the sub-study design is the same. The randomized controlled trial was designed to test the effect of a complete nutritional

supplement and the same nutritional supplement containing additional nutrients (prebiotics, probiotics and DHA) and given in addition to the daily food intake on growth (weight and height) and selected immune markers (C-reactive protein, faecal IgA and faecal calprotectin) in a cohort of HIV positive children aged 24-72 months (Figure 2.2). The clinical trial participants were randomly assigned to two study groups, namely, a group receiving the complete supplement, referred to as Group A and a group receiving the same complete supplement with added functional nutrients (prebiotics, probiotics and DHA), referred to as Group B.

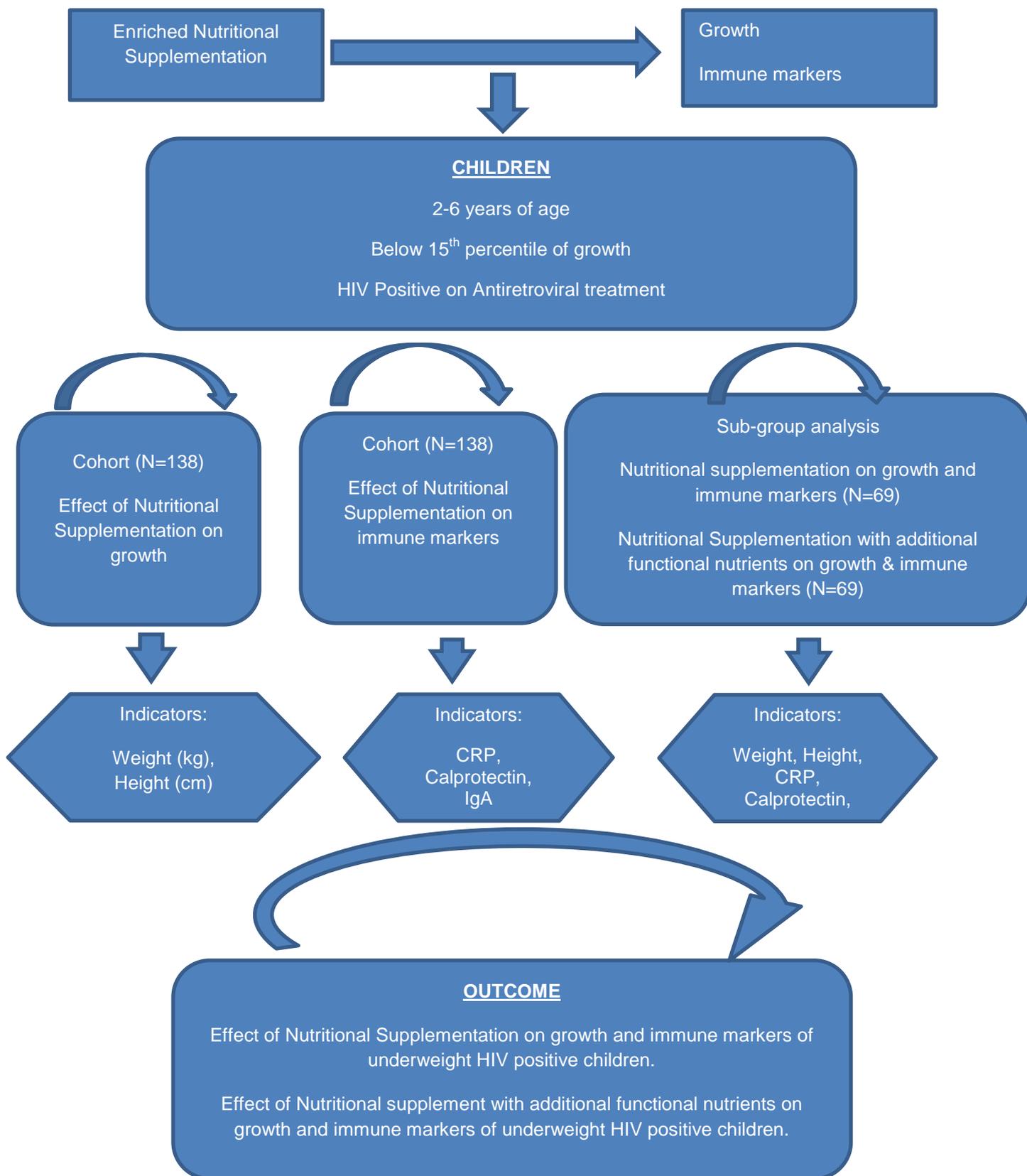


Figure 2.2: Conceptual Framework of Study design

2.6 RANDOMISATION

Study participants were assigned to one of the two groups stratified by age and gender. Stratified sampling is a technique whereby a population is divided into different sub-groups. A random sample is then drawn from the sub-group. Both males and females were included in the sub-groups. Age was sub-divided into the following age-groups; 2-3 years and 4-6 years. If more than one family member complied with selection criteria, they were assigned to the same treatment group for logistical reasons.

The randomization process was done with individual envelopes with two individual color codes. A block randomization was introduced based on an established list with balanced sequence to avoid any guess on treatment allocation. The Principle Investigator manually introduced participants in the database referred to as the trial balance system and each participant was randomized into Group A or group B.

2.7 SAMPLING

The study participants were sampled from the ARV treatment sites in all four sub-districts in the City of Cape Town Health district. The hospitals selected for recruitment sites included the HIV paediatric clinic of Tygerberg Academic Hospital, Groote Schuur Academic Hospital and Helderberg Hospital as well as primary health care facilities that are the drainage facilities to these secondary and tertiary hospitals, all based in the Cape Town City district. A drainage facility is a health facility that is within a 3-4km radius from a client's home. The drainage facility ensures the continuum of care and is the referral and support services for clients discharged from tertiary facilities. Establishment of drainage facilities are decided upon by the health authority based on population size and utilization rates per geographical area. Table 2.2 provides an overview of the relevant drainage facility, either clinic or community health centre, linked to the tertiary institution.

Table 2.2: Drainage primary healthcare facilities and ARV treatment sites linked to tertiary hospital in the City of Cape Town health district

Tertiary hospital	Drainage Primary health
Tygerberg Academic Hospital	Kraaifontein ARV clinic
Groote Schuur Academic Hospital	Crossroads ARV clinic Mitchells Plain ARV clinic
Helderberg Hospital	Eerste River ARV clinic

Participants were screened for eligibility at the relevant hospitals and invited to enrol. The Consent from parents, randomization and actual study activities took place at the study site based at the Tygerberg Academic Hospital.

2.8 INCLUSION AND EXCLUSION CRITERIA

Study participants were selected by criterion sampling involving the selection of cases that met pre-determined criteria. The criterion was developed based on a specific age-group and a specific CD4 count. The <6 year age-group was identified as this is the peak age for stunting/underweight. This <2years age-group was also old enough to drink a supplement and young enough (<6years) for caregivers to be able to manage and control the intake. The specific CD4 count level was identified as a disease intensity biomarker. The criteria are listed below.

- Age: 24-72 month old children.
- Gender: Males and females, all children were HIV positive as verified by the clinic records
- All ethnic groups were included.
- A minimum CD4 cell count of ≥ 500 for children older than 5 years or $\geq 15\%$ CD4 cell count for children younger than 5 years.
- Had no acute symptoms of infection (in the preceding two weeks) including TB.
- Absence or unstable end organ disease.
- Growth curve ≤ 15 th weight-for-age percentile of the Center for disease control/ National Centre for Health Statistics Child Growth Charts (2000).
- On antibiotics (All participants were routinely given antibiotics for duration of ART as prophylaxis; the continuous feeding with probiotics would support a better balance of intestinal microbiota).
- On antiretroviral medication as prescribed by the clinic.
- Written informed consent from the participant's legal representative.

Participants were excluded in the pre-screening phase and not considered for recruitment in the study. For the following reasons:

- Participants with unstable end-stage organ disease
- HIV+ children with a CD4 cell count <500 for children older than 5-years or <15% for children younger than 5-years.
- Acute gastrointestinal illness (diarrhoea, vomiting) or acute respiratory illness at the point of entry to the study.

- Participants who, on the basis of clinic records, would not be expected to comply with treatment. (Reasons included: frequently missed consecutive clinic appointments, non-compliance to other forms of treatment, e.g.:TB, and other reasons as documented in the study participant's clinic file.)
- On a dietary restriction
- Participating in another clinical trial during the last one month prior to the beginning of this study.
- Attending a day care facility where the supplement may not be correctly prepared for the participant
- Enrolled in the Provincial Government of the Western Cape Health Department's Nutrition Therapeutic Programme (NTP).
- Growth curve was >15th weight-for-age percentile on Center for disease control/ National Centre for Health Statistics Child Growth Charts (2000).

2.9 SUPPLEMENT DOSAGE, ADMINISTRATION AND QUALITY ASSURANCE

Study participants were provided with a 30 day supply (monthly) of the nutritional supplement over a period of 6-months. The supplement was administered via the oral route as a drink which was consumed either once a-day or twice a-day. A minimum of 250ml/day was needed to be consumed in addition to the usual diet. A target range for daily intake was determined by a pilot study and defined as a minimum of 250ml/day to a maximum of 500ml/day. The total energy content for a 500ml quantity of supplement was 500kcal based on 100kcal/100ml. Families of the children included in the trial were provided with a commercially available growing-up milk powder for siblings or relatives living in the same premises as the study participant. This was done to ensure that the participant consumed the required amount of supplement daily for 6-months and that no inappropriate sharing of the product with relatives occurred. At the end of the study period, all participants including siblings and relatives living on the same premises were provided with a similar growing-up milk powder for an additional period of 6-months that was not part of study investigation as was recommended by the Ethics Committee.

The supplement was provided in a powder form with clear instructions printed on the label of the tin. Parents/caregivers directly in charge of the child's meals and day-to-day activities were trained to reconstitute the powder as per manufacturer's instructions provided on the label and utilising the enclosed scoop. To ensure compliance, the parents/caregivers were asked to keep the empty supplement tins which would be handed in at the next monthly visit.

2.10 QUALITY ASSURANCE OF THE INTERVENTION

The manufacturer was responsible for quality assurance at the production stage and on product release. A certificate of quality assurance was provided and placed in the regulatory file. The internal quality control was based on the Codex Alimentarius standards (International Food standards FAO & WHO).

2.11 WITHDRAWAL FROM THE STUDY

The following criteria resulted in the participant being withdrawn from the study:

- Illness that required hospitalisation leading to interruption of treatment for more than 10 consecutive days or 20 non-consecutive days over the 6-month study period
- Participant was withdrawn on evidence of non-compliant consumption of supplement for 10 continuous days of non-use
- Severe infection that was diagnosed by a professional nurse or HIV treatment medical officer

On withdrawal whether voluntary or as directed by the Principle investigator, the following details were recorded:

- Type of illness
- Length of stay in hospital
- Non-consumption of the supplement and reasons thereof
- The weight of the child prior to hospitalisation

2.12 INTERVENTION DESIGN

Participants were examined monthly over a period of 6-months while receiving the intervention. Study visits occurred approximately at 22-25 days intervals. Visits were numbered according to the following system:

- Visit 0 = screening & randomization
- Visit 1 = end of 1st month
- Visit 2 = end of 2nd month
- Visit 3 = end of 3rd month
- Visit 4 = end of 4th month
- Visit 5 = end of 5th month
- Visit 6 = end of 6th month

2.12.1 Pilot study

A pilot study was carried out in 2006 in a group of children (n =10; 19 enrolled, 6 were lost to follow-up) aged 24-72 months attending the HIV clinic at Tygerberg hospital. The main

objectives of the pilot study was to determine the amount of a nutritional supplement (either Acidified milk powder {Pelargon} or Nutren Junior; {these supplements were not the ones used in the clinical trial}) that a child aged between 24 – 72 months could consume comfortably and consistently on a daily basis in order to determine volume tolerance. The minimum amount of supplement that was required to be consumed was 250 ml/day which was calculated to provide the additional energy intake to support weight gain.

The findings showed that the average daily consumption for the children drinking Perlagon or Nutren was 570ml per day and 646ml per day, respectively. The average weekly consumption for children drinking the Perlagon was 4208ml per week and for Nutren, 4457ml per week. From the children drinking Perlagon, 71% met the minimum requirement of 250ml/day. Of the children drinking Perlagon, 57% consumed > 400 ml/day. All (100%) children drinking Nutren Junior met the minimum requirement of 250 ml/day; 83% of children from the Nutren group drank >400ml per day.

Another finding, relevant to the design of the study and documented during the pilot, was the fear of stigma among mothers regarding the use of Pelargon. Mothers regarded this formula as appropriate for infants under the age of 6 months, especially used for HIV infected children. At that time, South Africa was using Pelargon as the infant formula of choice in the PMTCT (Prevention of Mother to Child Transmission) program, if the mother chose not to breastfeed. Therefore, many mothers/caregivers were reluctant to be seen with Pelargon in their possession. Mothers went as far as hiding the tins as soon as they received them, but they however reported giving the Pelargon to the child in the privacy of their homes.

The outcomes of the pilot defined two main aspects of the clinical trial:

1. A target range for daily intake was determined and defined as a minimum of 250ml/day to a maximum of 500ml/day.
2. The name of supplement was “supplement” to support compliance and ensure the success of the research study

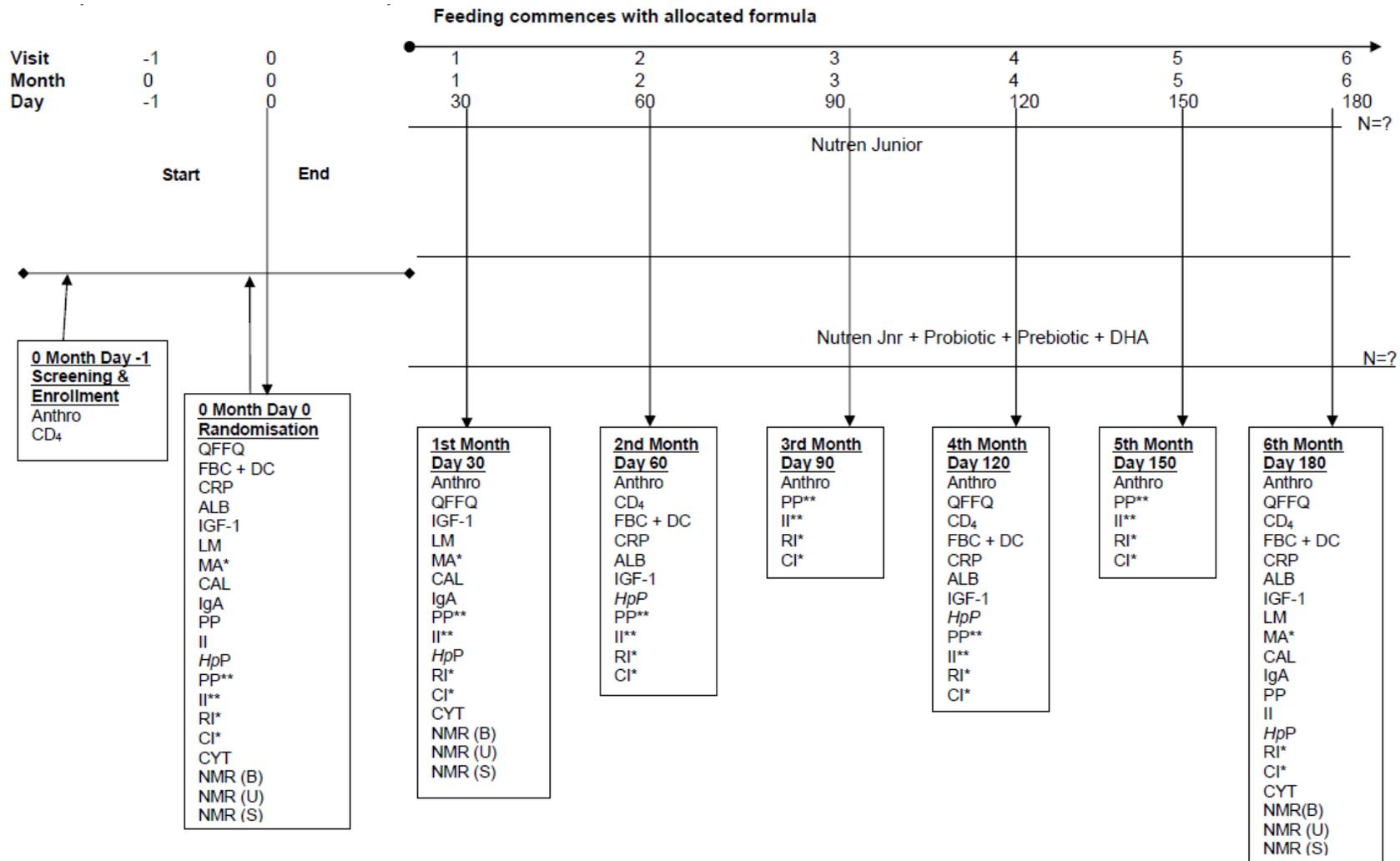


Figure 2.3: Intervention design of the main study

2.13 SCREENING AND ENROLMENT OF PARTICIPANTS

The screening and enrolment process was divided into three-main steps:

1. Pre-screening
2. Screening
3. Randomization

2.13.1 Pre-screening

On initiation at a new recruitment site, the Principle Investigator requested a list of all the children attending at the ARV clinic within the age-group of 24-72 months. The list contained each child's name, gender, folder number and contact details. The investigators then carried out folder reviews to confirm the pre-eligibility of a participant according to the pre-determined inclusion criteria, prior to the caregiver being approached by a researcher.

2.13.2 Screening

Once a child fulfilled the initial pre-screening eligibility, the parent or guardian was contacted telephonically or the investigators approached the parent/guardian at the child's routine monthly HIV clinic visit and provided them with an overview of the proposed study. If the parent/guardian was willing to allow their child to participate, they were invited to the main study site at the Tygerberg Academic hospital on a specified date.

The screening visit was identified as the initial visit (visit 0). The proposed study was explained to the parent/caregiver in detail according to ethical requirements. A PowerPoint presentation was used to explain in detail the proposed study to the parent/guardian (Appendix 1), if they agreed to allow their child to partake in the study. Thereafter the consent process was followed. The consent form (Appendix 2) was completed by the investigator with the guardian/parent of a minor and a witness in order to get permission to weigh the child, take a height measurement, and draw a blood sample, by a registered nurse with relevant experience. The weight and height measurement were used to plot a growth curve for each child to ensure their growth was \leq 15th weight-for-age percentile of the National Centre for Health Statistics Child Growth Charts. Blood samples were delivered to the Synexa Life Sciences laboratory based at Stellenbosch University Medical Campus, to determine the CD4 count. The parent/guardian was encouraged to wait for the outcome of the test, which took approximately 2-hours for the investigator to receive a result. While the parent/guardian and child waited, the site provided toys, child-based DVD's and magazines. Tea, coffee and cereal were provided for parent/guardian and child. If the result of the CD4 test fulfilled the inclusion criteria, the child was randomized to a treatment group, Group A or group B.

The randomization was carried out using the Trial Balance software (Sponsoring company's Research Centre, Switzerland). All supplement products were blinded by the manufacturer. The products contained the same product code, but tin labels were coloured Pink A and Green B to differentiate between the two separate treatment groups. The code was known only to the manufacturer and only opened at the end of the trial.

2.14 DATA COLLECTION

Data for anthropometric measurements and physiological fluid biomarker analysis over the study period (Table 2.3) were collected as follows:

Table 2.3: Data collection at visits 0 – 6

Test/Measure	Month of Testing						
	Screening & Rando	1 st mth	2 nd mth	3 rd mth	4 th mth	5 th mth	6 th mth
Visit n°	0	1	2	3	4	5	6
Weight and height	•	•	•	•	•	•	•
Age (in months) recording	•	•	•	•	•	•	•
C-reactive protein (CRP)	•		•		•		•
Calprotectin	•	•					•
Immunoglobulin A (IgA)	•	•					•
Food Frequency Questionnaire	•	•	•		•		•
Supplement Intake recorded	•	•	•	•	•	•	•

2.14.1 Anthropometry

The following anthropometric measurements were performed using standardised techniques as described in the literature (ISAK 2006).

2.14.1.1 Weight

The weight of the child was measured at each study visit. Weight was measured to the nearest 0.1kg, using an electronic load cell scale (UC-321 Personal Precision Health Scale, A & D Company, Ltd, Tokyo, Japan). The scale was placed on a flat hard surface. The measurements were carried out with the children wearing light clothing and with shoes removed. An average of 3 measurements were calculated and recorded. The scale was calibrated daily with an object of known weight. Appendix 3 provides the detailed standard operating procedure that was used by the investigators to weigh each participant.

2.14.1.2 Height

Height was measured at each study visit. Height was measured in a standing position, using a WHO approved SECA stadiometer. The height was measured to the nearest 0.1cm. An average of 3 measurements were calculated and recorded. See Appendix 4 which provides the detailed standard operating procedure that was used by the investigators to take a height measurement from each participant

2.14.2 Dietary intake

The Quantitative food frequency questionnaire (QFFQ) (Appendix 5) was completed at Visit 0, 1, 2, 4 and 6. The questionnaire was administered by a trained dietician. The questionnaire enquired about the food intake of the child for the previous month which required the parent/guardian to recall the child's intake. The dietician documented the supplement intake for the previous month. This was done through face-to-face interviews. The QFFQ was used as the dietary intake tool to establish a usual intake of the child over a set-period. The questionnaire also allowed for the documentation of precise food portion sizes in the form of specific household measures which is easier to define to the caregiver. The study aimed to establish a month's eating pattern and intake of a child, due to the supplementation being consumed over a month-to-month period. If the 24 hour recall method had been used, investigators would have only established the current consumption/1-day consumption which would not have been effective as the supplement was consumed over a month-to-month period⁽⁵¹⁾.

The MRC Food Finder booklet containing pictures of single food items and portion sizes was used to guide participants to estimate the quantities of the food and beverages consumed. Each interview lasted approximately 20-30 minutes to ensure that the investigator collects an accurate account of food intake and portion sizes. The Food Finder program includes in the computer software the South African Food Composition database, the information on food quantities which is derived from the MRC Food Quantities manual second edition. The program uses Recommended Dietary allowances as the reference standards, but also gives provision for including and using other dietary intake standards, including Dietary Reference intakes (DRI's) The Food Frequency questionnaire is displayed in Appendix 5 .

2.14.3 Biological samples

2.14.3.1 Blood samples

A 7ml sample of blood was drawn from a participant at visit 0, visit 2, visit 4 and visit 6 by a Professional Nurse. The Nurse would draw 7 millilitres (mL) of blood and place 4 mL into red top test tubes which contained no additives (Appendix 6). The sample was labelled and

transported to the Synexa Life Science laboratory for appropriate processing. At the laboratory the blood was spun down in a centrifuge to allow for a serum sample which is applicable for the CRP test. It was then re-labelled and stored at -80°C until analysis.

2.14.3.2 Faecal samples

Faecal samples were collected at visit 0, visit 1 and visit 6. Samples were collected by the research assistants on site within 30 minutes of evacuation and placed into a sterile collection tube. If the sample was not produced and collected on the day of investigations, the parent/guardian was trained to collect the faecal sample at home. The parent/guardian was responsible for collecting the sample within 30 minutes of evacuation. A 10g sample using a faecal collection and storage kit provided to the parent/guardian according to the protocol described in the Appendix 7. Samples were collected immediately after the children woke up in the morning, as the caregiver was instructed to collect the first faecal sample of the day. The faecal samples were kept in a cool environment/area of the home, if a refrigerator was not available, until the caregiver was ready to deliver the sample to the Laboratory at the end of the day.

For transportation to the laboratory, the research assistant placed the samples in a faecal kit box and delivered it to the laboratory. This occurred no more than 5 to 10 hours after faecal collection by the parent/guardian/research assistant. The faecal samples were delivered to the Synexa Life Sciences laboratory for processing (Appendix 9). The faecal samples were prepared for analysis according to the laboratories accredited procedures.

2.15 QUALITY CONTROL MEASURES FOR DATA COLLECTION

To ensure validity of all data collected, the following activities were implemented:

- All data collectors involved in the study underwent training implemented by the Principle Investigator to familiarise themselves with the study protocol. This process involved ensuring accuracy of collecting anthropometric data, techniques for collection of stool samples, appropriately administering the food frequency questionnaire and gaining useful techniques on probing the parent/guardian, without biasing the food recall process.
- Digital scale and stadiometer were calibrated on a daily basis using a known weight and known length marker.
- Prior to the start of the study, research team members collecting anthropometric data were expected to collect 50 weight and 50 height readings from children attending the HIV Paediatric Unit at Tygerberg Academic Hospital.

- The Laboratories analysing the blood and stool samples were both accredited by South African National Accreditation System (SANAS). SANAS is the only National body responsible for carrying out accreditations in respect of conformity assessment, as mandated through the Accreditation for Conformity Assessment, Calibration and Good Laboratory Practice Act (Act 19 of 2006). All quality assurance and accreditation documentation was placed in the regulatory file.
- The South African version food frequency questionnaire was piloted to ensure suitability and accuracy of food items specific to the population being accessed (i.e.: culturally acceptable food items). After the pilot study, the questionnaire was adapted (Appendix 5) for suitability purposes.
- Regular monitoring visits by the sponsor appointed research auditor was made during the study. Monitoring began with an initial visit prior to the studies commencement, to clarify all aspects of the protocol and documentation. The purpose of later visits during the implementation period was to evaluate study progress and adherence to protocol. The monitor checked Case Report Forms (CRF's) for completeness, clarity and consistency with the information in participants' files (source data checking). Any audit queries raised or recommendations made were addressed to the auditor's specifications. At the end of the trial the monitor made a final study closing visit to the site to ensure that all documentation was complete before being couriered to the manufacturer in Switzerland.

2.15.1 Research team

The research team consisted of:

Research team member	Qualifications	Data collection activities
Principle Investigator	Medical Doctor	Overall supervisory role
Co-Investigator	Registered dietician	Food frequency questionnaire
Co-Investigator	Registered dietician	Anthropometrics, faecal samples & Food frequency questionnaire
Research Assistant	Registered dietician	Anthropometrics, faecal samples & Food frequency questionnaire
Professional Nurse	Paediatric phlebotomist	Blood samples

All research team members attended a Good Clinical Practice Course presented at the Faculty of Medicine and Health Sciences, Stellenbosch University during 2008.

Co-investigators and research assistant were trained by the Principle Investigator according to the protocol. Co-investigators were also trained by the Principle Investigator according to the appropriate implementation of the program

2.16 ADVERSE EVENTS

An adverse event was defined as any untoward occurrence in a study participant who received the supplement irrespective of the causal relationship to the supplement (Appendix 9).

Adverse events included illnesses and/or reported signs and symptoms thereof (elicited or volunteered) occurring or worsening, and/or abnormal laboratory findings during the course of the study. Adverse events included occasions when the study participant contacted the investigator or their private physician and was examined or given medical direction. These may or may not have led to the withdrawal of the study participant from the study. Adverse events were classified as serious or non-serious according to accepted international criteria⁽⁵²⁾. The adverse events were documented according to the severity and reported to the Principle investigator and the Sponsor. See Appendix 10 for the adverse event documentation. An episode defined as a serious adverse event was reported to the Stellenbosch University Ethics Board within 24-hours of the event.

2.17 BREAKING OF THE CODE

The principal investigator received one of a set of sealed envelopes containing the product identification. In the event that the principal investigator identified an urgent need to break the code, the CRA/monitor and/or the Project Leader would have first been informed. All attempts to avoid breaking the code were made, but safety considerations prevailed at all times. All envelopes were retrieved at the end of the study.

2.18 ANALYSIS OF DATA

2.18.1 Data process

All data was recorded directly onto case report forms (CRF`s). The CRF`s were made of non-carbon paper providing three copies of each page. On completion of the study, 1 copy of the data collection page was removed from CRF and couriered to the Sponsor's Research Centre in Switzerland (NRC) where they were captured into an electronic database (Clin Trial 4.2). The remaining two pages were not removed from CRF. CRF`s have been archived for 10-years, as per ethical requirement's. Data was dual captured by trained data entry personnel. Validation was carried out by the data manager and queries referred back to Investigators. All queries had to be addressed, comments sent back to NRC, where data entry personnel entered the answers to the queries. The database then had to be approved by the data manager once all queries were addressed, a blind data review meeting was held, and the database was then locked and filed (See figure 2.3).

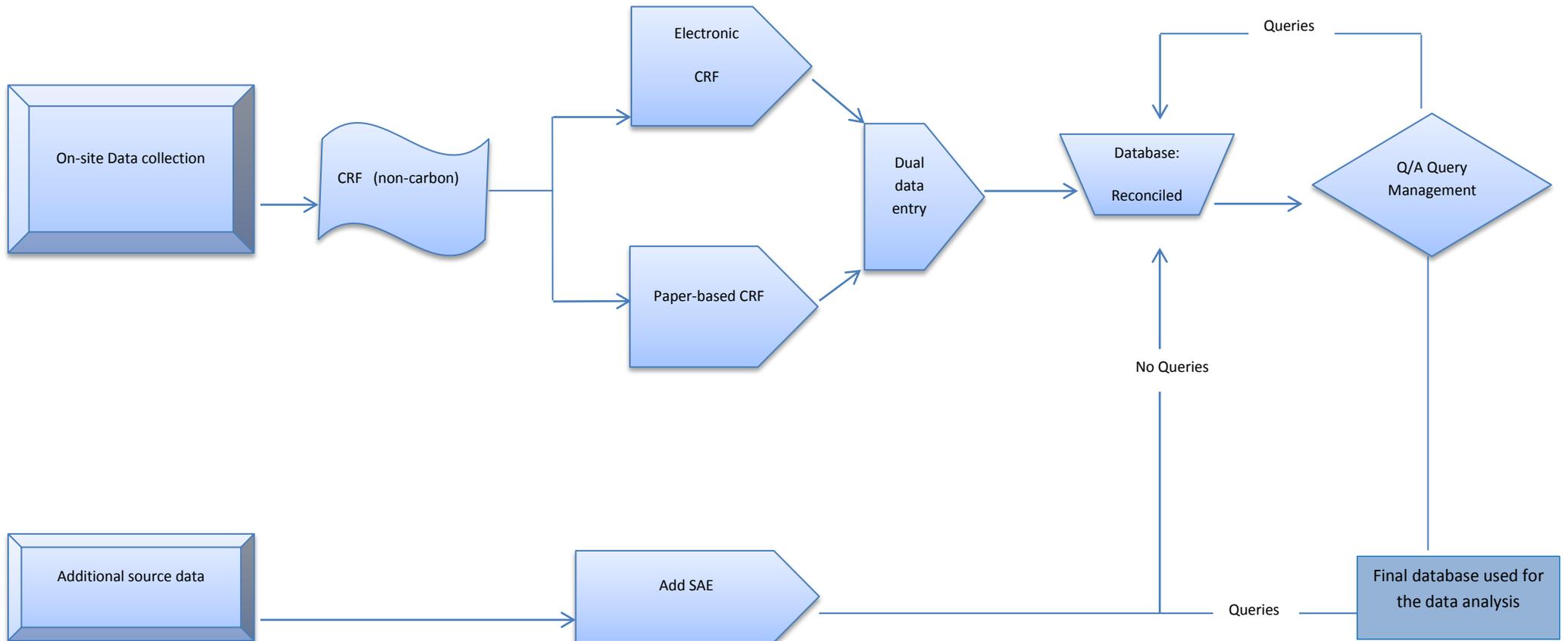


Figure 2.4: Research study data process (adapted from Sponsor`s clinical standard procedure)

2.18.2 Background to main study analysis

The primary purpose of the main trial was to demonstrate non-inferiority between two nutritional supplements for catch-up weight. As secondary measure, superiority between the two intervention groups on growth and biomarkers was considered.

The sample size calculation was calculated as follows: The alpha level was adjusted for two multiple tests. A change of $\Delta = 0.4$ in weight-for-age z-scores during a 6 month period, significant impact from baseline to 6-month in immune status and height were seen as clinically relevant. The standard deviation was extrapolated with $sd = 0.85$. A one-sided, one-sample superiority test ($\Delta > 0$) with an alpha level of $0.05/2=0.025$ and a power of 80% needed $n/\text{group} = 38$ children.

Since a change of $\Delta = 0.4$ would matter in practice, the non-inferiority boundary was chosen with 0.4. The standard deviation was extrapolated with $sd = 0.85$. A one-sided, two-sample non-inferiority test with an alpha level of $0.05/2 = 0.025$ and a power of 80% needed $n/\text{group} = 72$ children.

The sample size calculated for the clinical trial was calculated at 206 participants and a minimum of 144 participants to ensure 80% power with a 0.05 level of significance to support efficacy to establish non-inferiority.

2.18.3 Statistical analysis

The mixed linear regression analysis was used to analyse each parameter ie: anthropometric, immune markers and immune modulators over the 6-months and at each time point. P-values of < 0.05 were considered statistically significant.

2.18.3.1 Anthropometry

The effect of the enriched supplement on anthropometric status was determined by analysis of the cohorts measurements for weight and height expressed as z-scores. Height-for-age and weight-for-age were calculated using the WHO growth standards and the software programme, Anthro Plus. Screening was performed using the CDC/NCHS growth references. Analysis was carried out 3-years later and the new Anthro plus program was available to analyse the data. The Anthro plus software program was developed based on the WHO growth standards of 2007⁽⁵³⁾.

Certain aspects of the methodology used to create the two growth charts, namely, the WHO and the NCHS, were similar. However, it is important to consider the contextual difference of the two. The CDC growth charts are references. These charts showed how typical children from the US grew during a specific time period. The concern with this method is that typical

growth patterns may not provide the most accurate or ideal pattern for growth. In comparison, the WHO growth charts are standards, because they showed how a child should grow in optimal conditions^(54–57).

Studies have compared the difference in outcomes on underweight, wasting and stunting in children 0-60 months, when using the NCHS and the WHO growth curves and all studies have identified the same findings^(54–57). The prevalence of malnutrition was higher from age 0-6 months when the WHO growth standards were used instead of the NCHS growth reference, the difference became less obvious thereafter. At 1-year the prevalence of wasting was still more pronounced when the WHO standard was used and stunting prevalence showed similar results. The prevalence of underweight was found to be lower at 1-year when using the WHO growth standards. The WHO growth standard provides a more gradual increase in the prevalence of malnutrition in-comparison to the NCHS growth references which showed a sharp increase in the prevalence of malnutrition from 6-months onwards. However, from 2-years onwards to 5-years the WHO growth standard and the NCHS growth reference shows an incremental variation in prevalence. Table 2.4, describes why a small variation exists in the 2-5 year age bracket.

Table 2.4: Variation between the WHO Growth standards and the NCHS Growth References

WHO	NCHS
Growth standard is based on growth of breastfed infants	Growth reference based predominantly on growth of formula-fed infants
Based on more frequent measurements (every 2-weeks in the first 2-months and monthly thereafter)	Based on quarterly measurements (every 3 – months)

Based on these studies, the WHO growth standards are a better indicator of underweight and mortality due to underweight compared to the NCHS growth references^(54–57). Therefore the analyses using the WHO Anthro plus software program was deemed acceptable.

The raw anthropometry data was exported to the software program, Anthro Plus. Two records were excluded because one had a missing height, and one had both height and weight missing. The WHO anthropometric standards reference was used to evaluate the z-scores, to identify whether they are within the allowable range. For Height-for-age Z-scores, the z-scores smaller than -6 or greater than +6; and for Weight-for-Age Z-score, records smaller than -6 and for Weight-for-age Z-score greater than +5 were not considered. Comparisons were carried out with boxplots per age group over time. The SAS General linear models (GLM Procedure) was used, for each age group separately.

The z-scores have been derived from the WHO Multicentre Growth Reference Study (MGRS) of children’s measurements⁽⁵⁴⁾. The children in the MGRS study were fed and raised in environments that promoted optimal growth.

Z-score lines are either positive or negative depending on the distance from point 0 which is identified as the average. Generally, any point which is a distance from the average would represent a growth dimension. Other factors however, may be involved, these include: growth trend, health of the child and parents height. Table 2.5 provides an overview of the interpretation of the z-scores.

Table 2.5: Interpretation of Z-scores⁽³⁶⁾

Z-score	Growth Indicators			
	Length/height-for-age	Weight-for-age	Weight-for-length/height	BMI-for-age
Above 3	See note 1		obese	obese
Above 2			overweight	overweight
Above 1			Possible risk of overweight (see note 3)	Possible risk of overweight (see note 3)
0 (median/average)				
Below -1	Mildly stunted	Mildly underweight		
Below -2	Moderately stunted (see note 4)	Moderately underweight	wasted	wasted
Below -3	Severely stunted (see note 4)	Severely stunted (see note 5)	severely wasted	severely wasted

Notes:

1. A child in this range is very tall. Tallness is rarely a problem, unless it is so excessive that it may indicate an endocrine disorder such as a growth-hormone-producing tumour. Refer a child in this range for assessment if you suspect an endocrine disorder (e.g. if parents of normal height have a child who is excessively tall for his or her age).
2. A child whose weight-for-age falls in this range may have a growth problem, but this is better assessed from weight-for-length/height or BMI-for-age.
3. A plotted point above 1 shows possible risk. A trend towards the 2 z-score line shows definite risk.
4. It is possible for a stunted or severely stunted child to become overweight.
5. This is referred to as a very low weight in IMCI training modules. (Integrated Management of Childhood Illness, In-service training. WHO, Geneva, 1997).

Figure 2.5: Notes for z-score interpretation⁽³⁶⁾

2.18.3.2 *Biomedical*

The biochemical raw data was exported from Excel into the Stata 12 program. Descriptive analysis (mean, median, percentiles) was done for continuous and categorical data. Paediatric reference ranges from the National Health Laboratory Services and the Synexa Lifescience Laboratory were identified for calprotectin, CRP and IgA immune markers were utilised for analyses include cut off points here. Descriptive data was presented in frequency tables.

2.18.3.2.1 *CRP*

The ADVIA Chemistry system was used for the in vitro diagnostic to quantitatively determine CRP in human serum (Bayer's Healthcare). Polyethylene glycol (PEG) enhanced immunoturbidimetric was the method principle used. A specific antiserum was added to the serum sample to form a precipitate that could be measured turbidmetrically. A standard curve was constructed from the absorbances of standards. Concentrations were then determined.

The normal reference range for CRP in children is $\leq 10\text{mg}^{(58)}$. There is no official reference range for CRP for HIV positive children⁽⁵⁸⁾. Other studies have used 0- 8mg as a reference range for healthy children⁽⁵⁹⁾. For children with type 1 diabetes, 0.6mg/l was used as the reference range for CRP⁽⁶⁰⁾. In another study, for HIV positive mothers and HIV positive children the reference range $\leq 10\text{mg}$ was used⁽⁵⁸⁾. The National Health Laboratory (NHLS) in South Africa utilises the $\leq 10\text{mg}$ reference range for CRP in children (NHLS). For the purpose of this thesis, the reference range used for the analysis of CRP in this study was $\leq 10\text{mg}$.

2.18.3.2.2 *Calprotectin*

The PreventID CalDetect Assay Kit was used to determine in vitro, the concentration of calprotectin in the stool sample. The test device was removed from the packaging and 3-drops of the stool sample were placed at the sample opening which is on the right side of the test device. The result was read after 10 minutes. The number of colour bands appearing determined the concentration of calprotectin.

Table 2.6: Number of colour bands

Number of colour bands	Calprotectin concentration
1 colour band	Test run correctly, calprotectin not detected
2 colour bands	$\leq 15\text{ng/ml}$
3 colour bands	15-60ng/ml
4 colour bands	$>60\text{ng/ml}$
Control line not visible	Test invalid

Calprotectin reference range for children and adults >4 years of age have been identified as >50ug/l. The reference range utilised by the Synexa laboratories who carried out the calprotectin testing is >50ug/l (Synexa Life Sciences). Two studies (irritable bowel disease in children and HIV positive children on HAART) used >50ug/l as the reference range for HIV positive children^(29,61). A PhD dissertation by Hestvik in 2013 also used the reference range >50ug/l⁽⁶²⁾. One study considering gut and mesenteric lymph node involvement in paediatric patients infected with human immunodeficiency virus used 0-200ug/l as their reference range for HIV positive children because they utilised an enzyme-linked immunosorbent assay⁽⁶³⁾. For the purpose of this study the reference range >50ug/l was used.

2.18.3.2.3 *Immunoglobulin A*

The immunodiagnostic Assay was used to determine, quantitatively, the concentration of IgA in stool samples. A two-step incubation process was carried out on the stool sample to ensure all unbound substrates were removed. An acidic stop solution was added to the reaction. This resulted in a colour change from blue to yellow. The intensity of the yellow colour is directly proportional to the concentration of fecal IgA. A dose response curve was constructed based on the absorbance versus the concentration that was generated using results from the calibrators. Concentrations of IgA was then determined directly from the curve (Immunodiagnostik, Benshiem).

Synexa Life Sciences Laboratory utilise 510 -2040µg/ml as the reference range for IgA. For the purpose of this thesis, 510 – 2040 µg/ml was used as the reference for IgA (Synexa Life Sciences).

2.18.3.2.4 *Dietary intake*

The food intake data collected with the QFFQ was calculated as macronutrients and micronutrient intakes using the Food Finder, version 3 (MRC 2008). The Food Finder program was used for analysis based on the fact that it is a software program developed in South Africa and therefore based on South African food items. The software program calculates the mean portion sizes consumed. It then calculates the 16 food groups that are in the database and their contribution to the nutrient intake. The food items are coded. The codes link the food item to the specific food group, which then makes it possible for the calculation of the contribution of the different foods groups to the total energy and nutrient intake. From there the total intake can be printed out. The software program does not contain dietary intake levels for HIV infected children. The mean dietary intake per age-group was compared to a combination of nutrient based reference values, WHO Dietary Reference Intakes (DRI), namely the Estimated Average Requirement (EAR) and the

Recommended Nutrient Intake (RNI) reference ranges intake for children. The EAR was used because it represents an estimated median requirement which meets the requirements for a specified indicator of adequacy of half the healthy individuals in a group. The RNI represents the intake level that is estimated to meet the nutritional requirements of all the healthy individuals in a group^(64,65). Both measures were therefore well suited for this analysis.

Nutritional intake of the cohort was categorized into two age-groups, due to the assumption that children will eat different quantities of food at different age-groups. The two categories were 2-3 years and 4-6 years.

2.19 ETHICS REQUIREMENTS

Confidentiality of all study participants was considered a high priority and measures to ensure confidentiality were maintained throughout the study. No names were used on any documentation. Codes were assigned to identify each participant.

Parents /caregivers were provided with a thorough explanation of the proposed study and were given an opportunity to ask questions in their home language. A copy of the information sheet in the participant's home language was given to the parents/caregivers. Transport costs were provided to caregivers and participants based on the local public transport rates to the study site.

Written, informed consent was obtained from each parent/caregiver on behalf of the study participant prior to enrolment in the study. The consent form was signed and dated by the study participants' legal representative and the research assistant. The consent form was completed in three copies: the first copy was kept in the investigator's file, the second in the study participant's notes, and the third copy was given to the parent/caregiver. Both investigators enrolling participants were bilingual (able to communicate in English or Afrikaans), which ensured clear communications between investigators and Afrikaans speaking families. Families whose home-language was isiXhosa, were either able to communicate in English with the investigator or they brought along a family member to translate or an isiXhosa-speaking Nurse in the clinic was requested to translate. Where translators were needed, the consent form was clearly signed by the translator.

No study participant received the supplement before completion of the written informed consent.

Investigators adhered to the guidelines regarding allowable blood volumes for research on participants who are children. According to the Health Research Ethics Committee (HREC),

blood volume should not exceed 3% of the total blood volume during a time period of 4 weeks; and blood volume should not exceed 1% of the total blood volume at any single time;

Note: total blood volume is estimated at 80 to 90 ml/kg body weight; 3% is 2.4 ml blood per kg body weight⁽⁶⁶⁾.

Investigators drew a maximum of 7ml of blood at the following visits: randomisation, visit 1, 2, 4 and 6. There was a maximum period of 2months and minimum period of 4-weeks between blood collections. No blood was drawn at visit 3 and visit 5.

Post-study completion, participants and siblings receiving the supplement were weaned-off the supplement over a period of 6-months. At 6-months all participants and siblings were referred to the Nutritional Therapeutic Programme at their local health facility

The study was approved by the Committee for Human Research of the Faculty of Medicine and Health Sciences at Stellenbosch University (Ref nr: N/05/10/18). The Committee was notified of all subsequent additions or changes in the study protocol. Notification was provided to the Committee in the case of an adverse event or Serious Adverse Event during the study. A yearly progress report was submitted to the Ethics Committee. The Ethics approval letter is attached in Appendix 11.

CHAPTER 3: RESULTS

3.1 SAMPLE DESCRIPTION

Five ARV facilities provided the investigators with a list of children attending each facility for HIV care. These lists included children in the age-group 24-72 months. The potential pool for recruitment consisted of 633 children. One hundred and sixty one (161) children did not fit the inclusion criteria; while 327 children were unreachable due to either not arriving for their scheduled clinic visit, or the contact phone details provided were no longer valid. One caregiver refused the enrolment of their child to take-part in the study. One hundred and forty four (144) children were recruited, enrolled and randomized. Five (5) children were excluded due to inconsistencies of the medical folders at the clinic. Medical folder inconsistencies included:

- Children attending at different facilities for other medical conditions such as TB
- Children who were commenced on TB treatment were seen at TB care facilities that were not integrated with HIV services, which resulted in investigators not being fully aware of the child's medical profile according to their TB care
- Children were being seen by specialist tertiary institutions and their medical folders at the ARV clinic site was not updated with the details from the tertiary institution.

There were no drop-outs of study participants during the period of data collection. The final sample consisted of 138 HIV-positive children between the ages of 24 and 72 months (Figure 3.1).

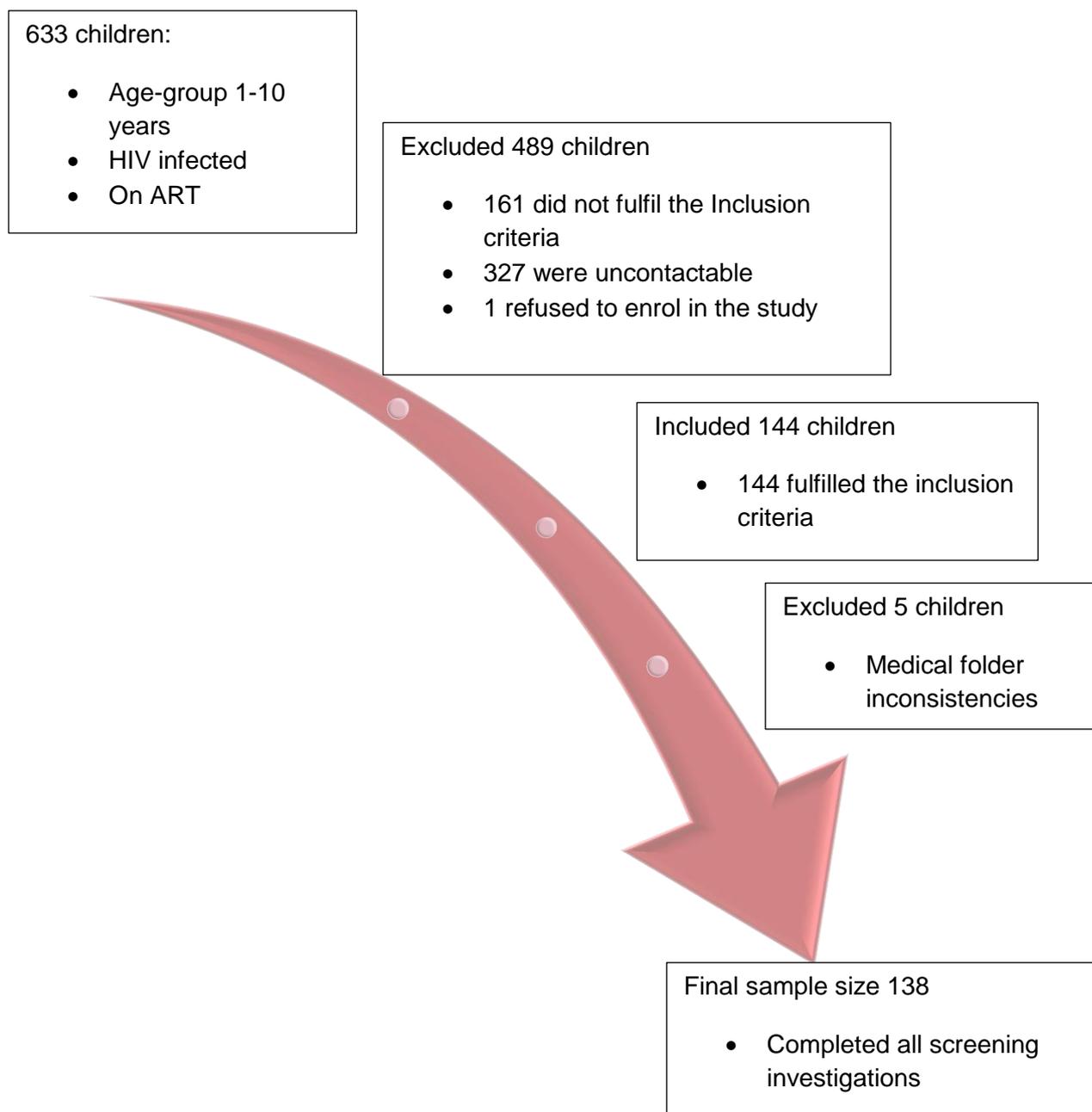


Figure 3.1: Flow diagram depicting the recruitment process and the total number of participants enrolled and included in the study

The total number of HIV-positive children included in the study was 138 (64 boys and 74 girls). Sixty nine (n=69; 50%) children were in the age category 2-3 years and 69 (50%) in the age category 4-6 years. Children were enrolled predominantly from Tygerberg Hospital ARV site (39.1%) and Crossroads Community Health Centre (25.3%), while a small proportion (17.3% and 18.1%) enrolled from the Helderberg and Grooteschoor ARV sites

respectively (Table 3.1). From the children included in the study, only 11% of the households they resided in had a fridge.

The mean energy intake for the 2-3 year age-group was 6542kJ which is more than 100% of the DRIs for that age-group. The mean intake for the 4-5 year group was 7336kJ which is >100% of the DRIs.

In this study, 45% of participants were <5th percentile for WFA¹ (underweight) and 54% were between the 5th and 15th percentile WFA² (mild-moderate underweight). According to HFA, 82% of the participants were <5th percentile³ (stunted) and 17% were between the 5th and 15th percentile⁴ (mild-moderate stunting).

After conversion of percentiles to weight-for-age z-scores and height-for-age z-scores, 84% of the children were mildly underweight, and 2.9% were severely underweight. Nine five percent (95%) of children were mildly stunted, and 26% were severely stunted.

The median CRP for the participants was 18mg/l. The reference range for CRP was <10mg/l (Synexa Lifescience); study participants therefore had a median CRP above the reference range. The median calprotectin value for the participant's was 127ug/g. A reference of 50ug/g⁽²⁹⁾ was used for calprotectin. Study participants had a median calprotectin value above the reference range. The median IgA value for the participants was 6624ug/ml. A reference range of 510-2040ug/ml was used for analysis. Study participants had a median IgA value above the reference range. All 3 biomarkers, namely CRP, calprotectin and IgA were above the reference ranges which are indicative of an inflammatory environment.

Table 3.1: Demographic detail of study participants by gender, recruiting site, age, dietary intake, anthropometry and biomarkers

Characteristic	Characteristic defined	Frequency (n=138)	% (n=100%)
Gender	Male	64	46
	Female	74	54
Recruitment ARV hospital	Groote Schuur Hospital	25	18.1
	Helderberg Hospital	24	17.3
	Tygerberg Hospital	54	39.1

1 A percentile value below the 5th centile for weight-for-age (WFA) indicates true undernutrition in a child

2 A percentile value between the 5th and 15th centile for weight-for-age (WFA) indicates mild-moderate undernutrition in a child

3 A percentile value below the 5th centile for height-for-age (HFA) indicates stunting in a child

4 A percentile value between the 5th and 15th centile for height-for-age (HFA) indicates mild-moderate stunting in a child

Age group (yrs)	Crossroads CHC	35	25.3	
	2	40	29	
	3	29	21	
	4	38	28	
	5	31	23	
Fridge**	No. of households that have a fridge	15	11	
	No. of households that do not have a fridge	123	89	
Dietary Intake**	2-3 year (mean)	6542kJ	>100*	
	4-5 year (mean)	7336kJ	>100*	
Anthropometry**	<5 th percentile (WFA) (Defined as underweight)	63	45	
	>5 th – 15 th percentile (WFA) (Defined as mild-moderate underweight)	75	54	
	<5 th percentile (HFA) (Defined as stunted)	113	82	
	>5 th - 15 th percentile (HFA) (Defined as mild-moderate stunting)	24	17	
	<-1SD (WAZ) (Defined as mild underweight)	116	84	
	<-2SD (WAZ) (Defined as moderate underweight)	36	26	
	<-3SD (WAZ) (Defined as severely underweight)	4	2.9	
	<-1SD (HAZ) (Defined as mild stunting)	132	95	
	<-2SD (HAZ) (Defined as moderately stunted)	95	68	
	<-3SD (HAZ) (Defined as severely stunted)	36	26	
	Biomarker**	CRP [#] (median)	21	18mg/l
		Calprotectin [#] (median)	22	127ug/g
IgA [#] (median)		17	6624ug/ml	

*Dietary Reference Intake: 2-3 years 2550kJ; 4-6 years 3000kJ

** Data pertaining to baseline data

#Reference range: CRP <10mg/l⁽²⁹⁾; calprotectin <50ug/g (Synexa Lifesciences); IgA 210-5040ug (Synexa Lifesciences)

Dietary intake ranges were taken from the Reference Nutrient Intakes (RNI) for children, for the age-groups 2-3 years and 4-6 years. Table 3.2 provides a summary of the dietary intake of energy, macronutrients and selected micronutrients of the children, at visit 0. At visit 0 the children were not consuming the enriched supplement. The totals are expressed as means.

The footnote provides details on the Dietary Reference Intake (DRI) per specific nutrient by age-group. An additional footnote describes Nutrition recommendations.

When comparing the dietary intake of the children against the DRI for that age-group at visit 0, the 2-3 year age-group children were consuming above the required reference range for intake of nutrients. For specific macronutrients such as carbohydrates and proteins, the group consumed approximately double the reference ranges (207g vs 143g; 49g vs 14g respectively). For micronutrient`s such as magnesium, riboflavin, thiamine, vitamin A and zinc, the group consumed approximately double the required reference intake ranges. For folate and calcium, a marginally higher intake was seen when compared to the DRI (168mcg vs 150mcg; 567mg vs 500mg respectively). Iron intake was similar to the DRI for iron for the 2-3 year age-group.

In the 4-6 year age-group, the intakes for calcium (530mg vs 800mg), iron (8.5mg vs 10mg) and folate (177mcg vs 200mcg) were below the reference standard for these nutrients. Energy, carbohydrates, protein and fat intakes were all above the reference range. Vitamin A intake was substantially higher (973mcg vs 400mcg) than the DRI for this group.

Table 3.2: Mean dietary intake of the cohort at visit 0

Visit No. Nutrient	Visit 0	
	2-3 years**	4-6 years***
	Total	Total
Energy (kJ)	6542.5	7336.6
Carbohydrate (g)	207.9	237.9
Total protein (g)	49.3	54.9
Total fat (g)	52.2	55.6
Ca (mg)	567.4	530.0
Fe (mg)	7.4	8.5
Folate (mcg)	168.4	177.4
Mg (mg)	196.6	220.3
Niacin (mg)	11.7	13.7
Riboflavin (mg)	1.7	1.8
Thiamin (mg)	0.7	0.9
Fe (mg)	7.4	8.5
Vitamin A (RE) (mcg)	948.0	973.6
Zn (mg)	6.7	7.4

**DRI's 2-3 year old children: Energy 2500KJ; CHO 143g; Total Protein 14g; Total fat 33g-44g; Ca 500mg; Fe 7mg; Folate 150mcg; Mg 80mg; Riboflavin 0.5mg; Thiamine 0.5mg; Vitamin A 300mcg; Zn 3mg

***DRI's 4-6 year old children: Energy 3000KJ; CHO 143g; Total Protein 20g; Total fat 27g-38g; Ca 800mg; Fe 10mg; Folate 200mcg; Mg 130mg; Riboflavin 0.6mg; Thiamine 0.6mg; Vitamin A 400mcg; Zn 5mg

DRI's based on nutritional requirements for healthy children.it is however, recognized that energy is increased in HIV positive children^(39,67).

3.2 ANALYSIS OF OBJECTIVE 1

The initial study report showed that there was no major and significant differences between the two treatment groups of the cohort for the primary outcome, i.e. catch-up growth as well as secondary's biomarkers measured. The aim of this sub-study was therefore to combine and analyse the data for the whole cohort in order to better determine the effect of a complete nutritional supplement and given in addition to the daily food intake on the growth (height and weight) in underweight HIV positive children aged 24-72 months.

3.2.1 Dietary intake

Table 3.3 lists the dietary intake of the children at visit 0, 4 and 6. At visit 0, the children were not consuming the enriched supplement. At visit 4 and 6, the children were consuming the enriched supplement.

From visit 0 to visit 4 the 2-3 year age-group had a mean total energy intake change of approximately 1000kJ. Calcium, vitamin A, and zinc increased substantially from visit 0 to visit 4 (567mg vs 825mg; 948mcg vs 1391mcg; 6.7mg vs 10mg respectively). From visit 4 to visit 6, the nutrient quantity continued to increase. The 4-6 year group of children showed a similar increase from visit 0 – visit 4. The mean energy increased by 1000kJ. Calcium, vitamin A, folate and iron increased substantially (530mg vs 870mg; 973mcg vs 1575mcg; 177mcg vs 268mcg; 8.5mg vs 13mg respectively) over the period from visit 0 to 4.

The nutritional supplement provided, increased the nutritional intake for the children in both age-categories.

When comparing the dietary intake analysis against the DRI, the 2-3 year age-group experienced marginal increases in nutrient intake overall, and the intake was above the reference standard. The greatest increases were noted in calcium (increase 258g), vitamin A (increase 443 mcg) total protein (increase 32g) and total fat (increase 9g).

The 4-6 year age-group in visit 0 had nutrient levels below the DRI (Ca, Fe and folate). At visit 4 these nutrients improved to above the DRI reference. Overall the nutritional supplement improved nutrient intake at visit 4 for this age-group. The greatest increase was seen in energy (1461kj increase), fat (18g increase) and vitamin A (601mcg increase). For some other micronutrients such as magnesium, niacin, zinc and riboflavin an increase was also observed.

At visit 6 the children were still consuming their daily intake and the nutritional supplement. For the 2-3 year age-group, nutrients continued to increase overall. Macronutrients (energy, protein, fat) and micronutrients (calcium, iron magnesium, niacin, riboflavin, iron vitamin A)

all remained substantially higher than the DRI. This indicated optimal intake of supplement and daily dietary intake.

The 4-6 year age-group data showed that protein, niacin, riboflavin, thiamin, vitamin A and zinc still increased. Although macronutrients (protein and fat) and micronutrients (zinc, vitamin A, thiamine, folate, riboflavin, magnesium, iron and calcium) decreased in comparison to visit 4, the nutrients still remained above the DRIs.

Including the supplement into the children's dietary intake resulted in improvements in macronutrient and micronutrient intakes for both age-categories.

Table 3.3: Dietary Intake of the cohort at visit 0, 4 and 6 by age-group

Visit No.	Visit 0		Visit 4*		Visit 6*	
	2-3 years** (n=69)	4-6 years*** (n=69)	2-3 years** (n=69)	4-6 years*** (n=69)	2-3 years** (n=69)	4-6 years*** (n=69)
Nutrient	Total	Total	Total	Total	Total	Total
Energy (kJ)	6542.5	7336.6	7429.1	8797.4	7719.8	8722.6
Carbohydrate (CHO) (g)	207.9	237.9	237.7	275.8	241.6	282.2
Total protein (g)	49.3	54.9	52.9	65.2	56.5	61.6
Total fat (g)	52.2	55.6	61.8	73.5	66.2	70.5
Calcium (Ca) (mg)	567.4	530.0	825.2	870.2	848.4	861.3
Iron (Fe) (mg)	7.4	8.5	10.9	13.1	11.6	12.9
Folate (mcg)	168.4	177.4	233.8	268.4	229.5	256.7
Magnesium (Mg) (mg)	196.6	220.3	215.8	257.6	225.0	252.3
Riboflavin (mg)	1.7	1.8	1.9	2.2	1.9	2.1
Thiamin (mg)	0.7	0.9	0.9	1.1	1.0	1.1
Vitamin A (RE) (mcg)	948.0	973.6	1391.6	1575.6	1403.0	1489.9
Zinc (Zn) (mg)	6.7	7.4	10.0	11.5	10.6	11.2

* Includes intake from supplement

**DRI's 2-3 year old children: Energy 2500KJ; CHO 143g; Total Protein 14g; Total fat 33g-44g; Ca 500mg; Fe 7mg; Folate 150mcg; Mg 80mg; Riboflavin 0.5mg; Thiamine 0.5mg; Vitamin A 300mcg; Zn 3mg

***DRI's 4-6 year old children: Energy 3000KJ; CHO 143g; Total Protein 20g; Total fat 27g-38g; Ca 800mg; Fe 10mg; Folate 200mcg; Mg 130mg; Riboflavin 0.6mg; Thiamine 0.6mg; Vitamin A 400mcg; Zn 5mg

3.2.2 Weight

Weight measurements were performed monthly and plotted against CDC/NCHS growth references (percentile data). Data was then analysed by converting percentile data to z-scores. Z-scores for the cohort was categorized according to <-1SD (mildly underweight), <2SD (moderately underweight) and <-3SD (severely underweight).

The comparison of relative increases in weight overtime is presented in Table 3.4. This table shows the absolute weight change overtime. The table shows that the greatest increase in weight from visit 0 to visit 6 was evident in the 2-year age-group for both boys and girls (1.6kg; 1.7kg respectively) and the 5 year age-group, both boys and girls (2.2kg; 1.9kg respectively). The 3 and 4 year old age-group's only experienced a marginal increase for boys (1.5kg; 1.3kg respectively) and girls (0.7kg; 0.9kg respectively).

Table 3.4: Mean weight (kg) of study participants by age and gender

Visit No.	0		1		2		3		4		5		6		Statistical significance
Gender	M (n=16)	F(n=18)	M (n=14)	F(n=17)	M (n=14)	F(n=16)	M (n=12)	F (n=15)	M(n=11)	F (n=15)	M (n=11)	F (n=14)	M (n=9)	F (n=14)	
2-year*	10.8	10.5	11.5	11.0	11.6	11.2	11.9	11.5	12.2	11.6	12.5	12.0	12.5	12.2	p<0.000
3-year*	12.1	12.0	12.8	12.4	12.9	12.3	13.2	12.4	13.3	12.6	13.4	12.7	13.6	12.7	p<0.529
4-year*	14.2	13.8	14.6	14.2	14.9	14.5	15.0	14.5	15.1	14.6	15.4	14.6	15.5	14.7	p<0.001
5-year*	15.3	14.6	15.7	15.0	15.8	15.8	16.1	15.8	16.4	16.0	16.8	16.3	16.8	16.5	p<0.001

*each child that was measured for weight over the 6-month duration had 6 measurements. Therefore the accumulated total sample size on this table is greater than the sample size (138).

Figures 3.2-3.3 reflect the pattern of growth from visit 0 – visit 6 by gender. It shows that both boys and girls had improved weight gains from visit 0 – visit 6.

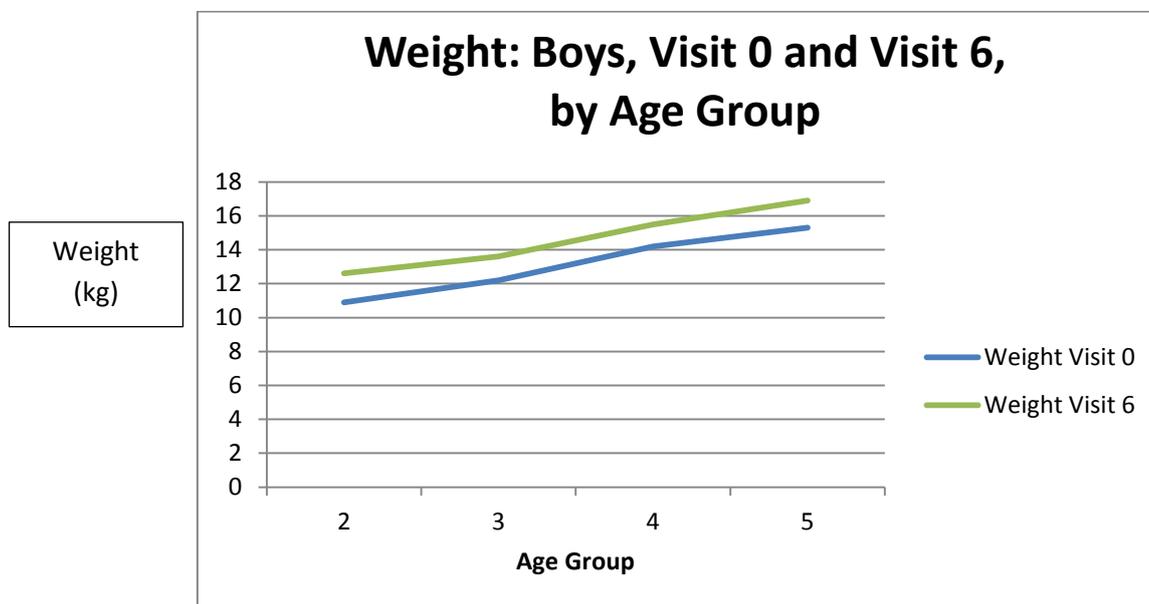


Figure 3.2: Weight gain from visit 0- visit 6 by age-group for boys

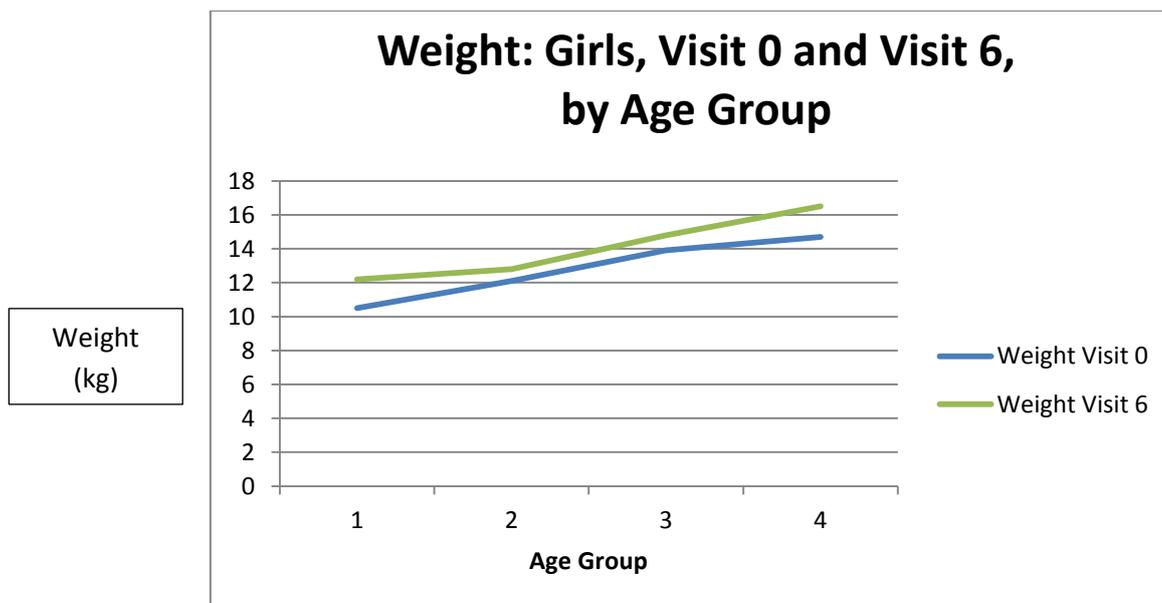


Figure 3.3: Weight gain from visit 0 – visit 6 by age-group for girls

Figures 3.4 – 3.6 present the comparison with boxplots per age group over time. The F-test was > 0.05 (10.46; 3.63; 5.44 respectively), which indicates that all mean values for all the visits were similar for each age-group. The effect of the supplement on growth was more pronounced in younger children. For age-groups 2, 4 and 5 the absolute weight gain values were significant ($p < 0.000$; $p < 0.001$; $p < 0.000$ respectively)

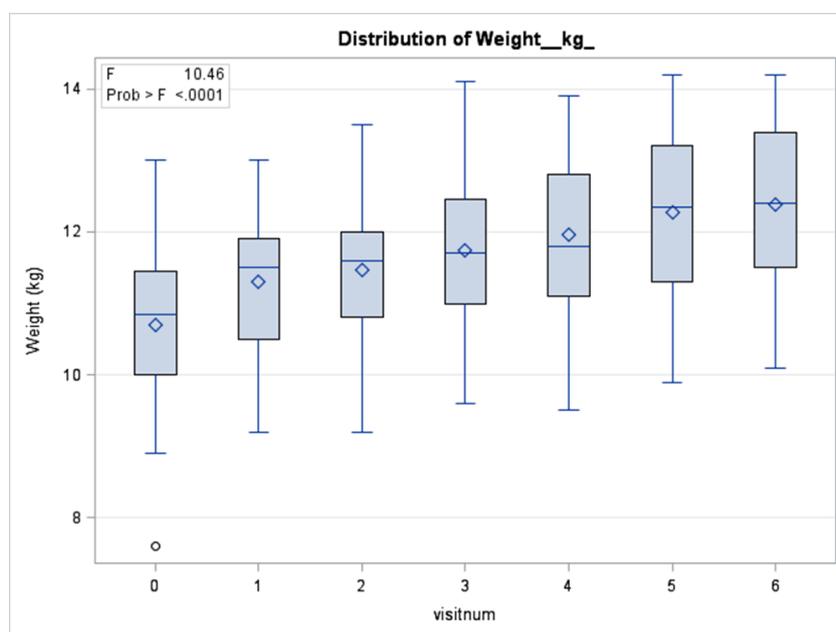


Figure 3.4: Mean absolute weight visit 0-6 by age (2-years)

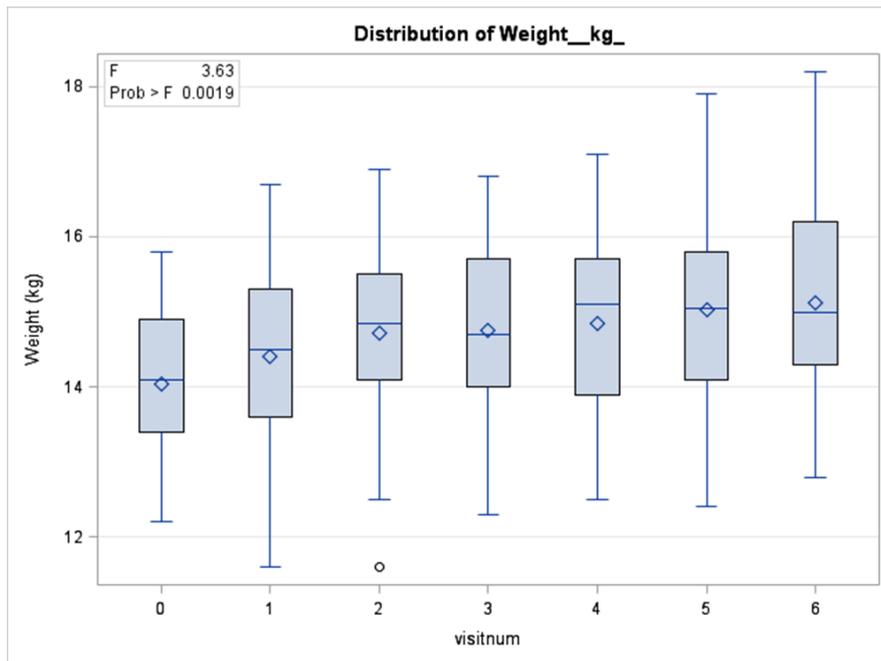


Figure 3.5: Mean absolute weight visit 0-6 by age (4-years)

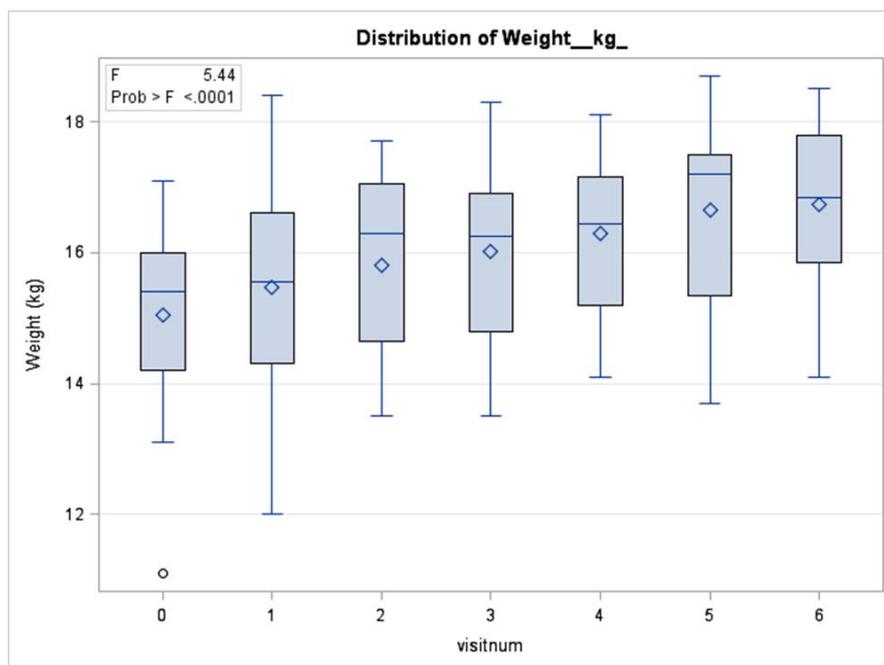


Figure 3.6: Mean absolute weight visit 0-6 by age (5-years)

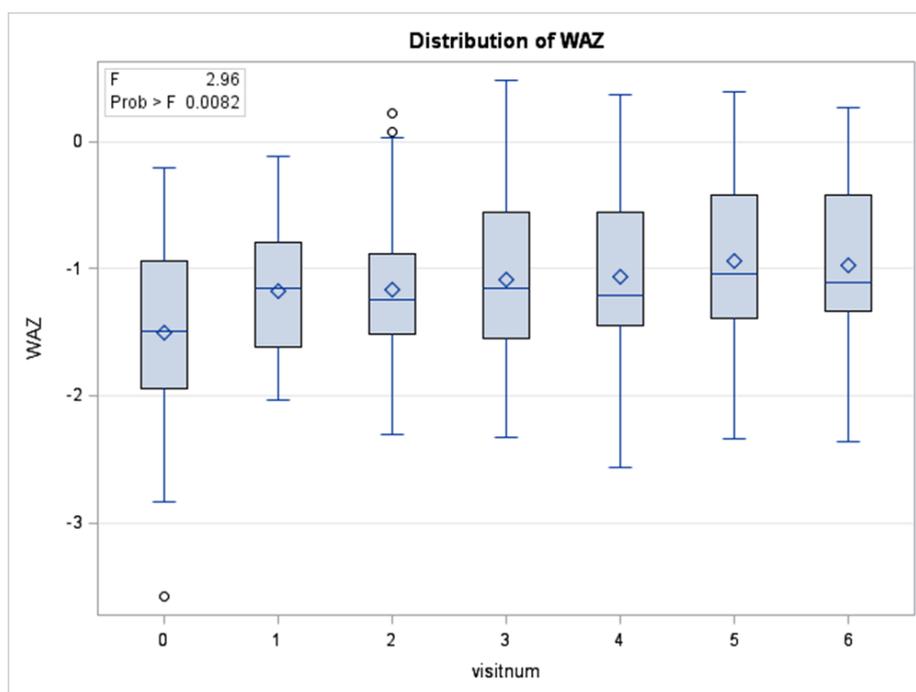
Table 3.5 shows the z-score data disaggregated by gender and age. For the 6-months duration of the intervention, the cohort remained within the moderately underweight category, except for girls in the 2-year age-group. This group was initially moderately underweight, but after 6-months of supplementation they moved to the mildly underweight category. There was an average incremental improvement over time for both boys and girls of -0.28.

Table 3.5: WAZ of study participants at visit 0 and visit 6 by age and gender

Gender	M* (n*=19;10)	F* (n=21;12)	M (n=10;9)	F (n=19;15)	M (n=17;9)	F (21;13)	M (n=18;15)	F (n=13;14)
Age-group	2		3		4		5	
Statistical significance	p<0.0082		p<0.967		p<0.864		p<0.138	
WAZ* Visit 0	-1.65	-1.37	-1.81	1.58	-1.51	-1.58	-1.83	-1.88
WAZ Visit 6	-1.08	-0.86	-1.19	-1.66	-1.28	-1.58	-1.5	-1.32
Interpretation	moderately underweight	moderately underweight - mildly underweight	moderately underweight					

*M: male; n: sample size; F: female; WAZ: weight-for-age Z-score

The F-test = >0.05 (2.96), which indicates that all mean values for all the visits were similar for each age-group. The effect of the supplement on growth was more marked in younger children. For age-group 2-years (figure 3.7), weight-for-age z-scores improved from visit 0 – visit 6 (p<0.0082). For age-groups 3, 4 and 5 the weight-for-age z-scores were not statistically significant.



*WAZ: weight-for-age Z-score

Figure 3.7: Mean WAZ visit 0-6 by age (2-years)

Data was further analysed to identify whether weight improved in the children who were severely underweight compared to the children who were mildly to moderately underweight. According to table 3.6 the children who were below the 5th percentile for WFA experienced

the greatest improvement in weight gain from visit 0 to visit 6 ($p < 0.009$) compared to the children who were between the 5th -15th percentile for WFA. When comparing the z-scores, at visit 0, 84% of children had a z-score below the $< -1SD$ and 26% had a z-score $< -2SD$. After 6 months of supplementation, 68% had a z-score $< -1SD$ and 13% had a z-score $< -2SD$. Weight change over time (visit 0 – visit 6) was statistically significant for the $< -1SD$ and $< -2SD$ ($p < 0.003$; $p < 0.010$ respectively), but not statistically significant for the children $< -3SD$ ($p < 0.737$). Although not statistically significant, a clinical improvement in weight gain was evident for children with weights $< 3SD$ at the outset of the study. Four (4) children were $< -3SD$ at visit 1 and after 6 months of supplement only 1 child was $< 3SD$ for weight.

Table 3.6: Chi-square test comparing children $> 5^{th}$ - 15th percentile and $< 5^{th}$ percentile between visit 0 and 6 and z-scores at visit 0 and visit 6

Characteristics	Cohort		Statistical significance
	Visit 0 (n=138)	Visit 6 (n=104)	
Visits			
$< 5^{th}$ percentile:	63 (45%)	32 (30%)	$p < 0.009$
$> 5^{th}$ percentile:	75 (54%)	72 (69%)	
Z-score: WAZ			
$< -1SD$	116 (84%)	71 (68%)	$p < 0.003$
$< -2SD$	36 (26%)	14 (13%)	$p < 0.010$
$< -3SD$	4 (2.9%)	1 (0.96%)	$p < 0.737$

Figures 3.8 – 3.9 depicts the growth by z-scores for boys and girls. For boys, the pattern shows a linear improvement in weight overtime. All of the boys at visit 0 had a WAZ below -1.5, after 6-months of supplement, they all appeared between the -1 and -1.5 WAZ. The girls also experienced improved weight gain over the 6-month period. The improvement was more pronounced in the 2 and 5 year age-groups.

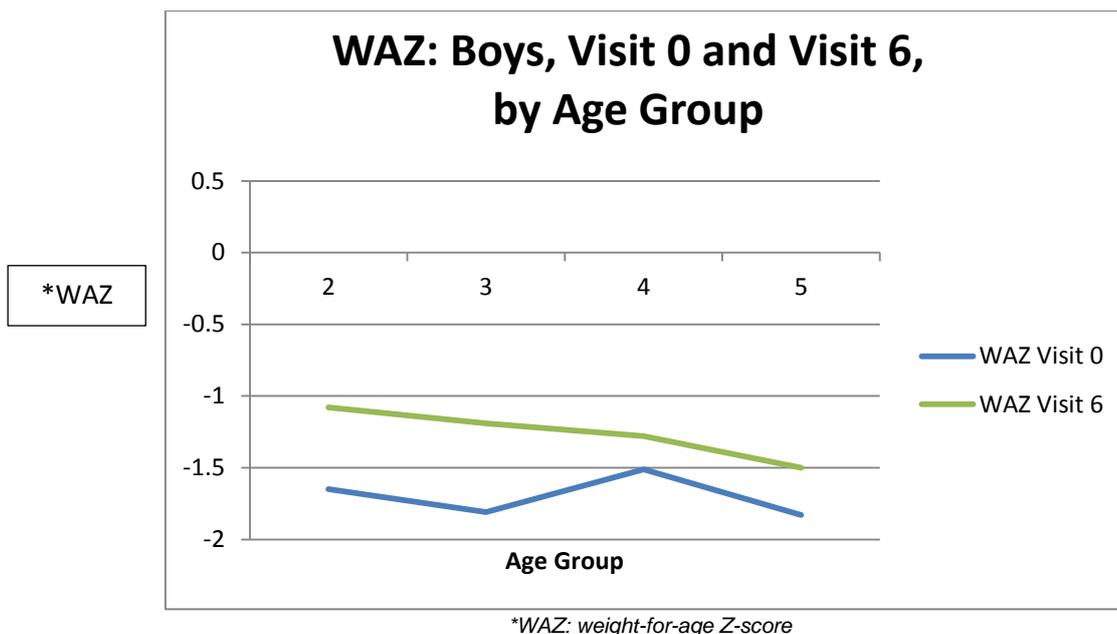


Figure 3.8: Weight-for-age z-score at visit 0 and visit 6 by age-group for boys

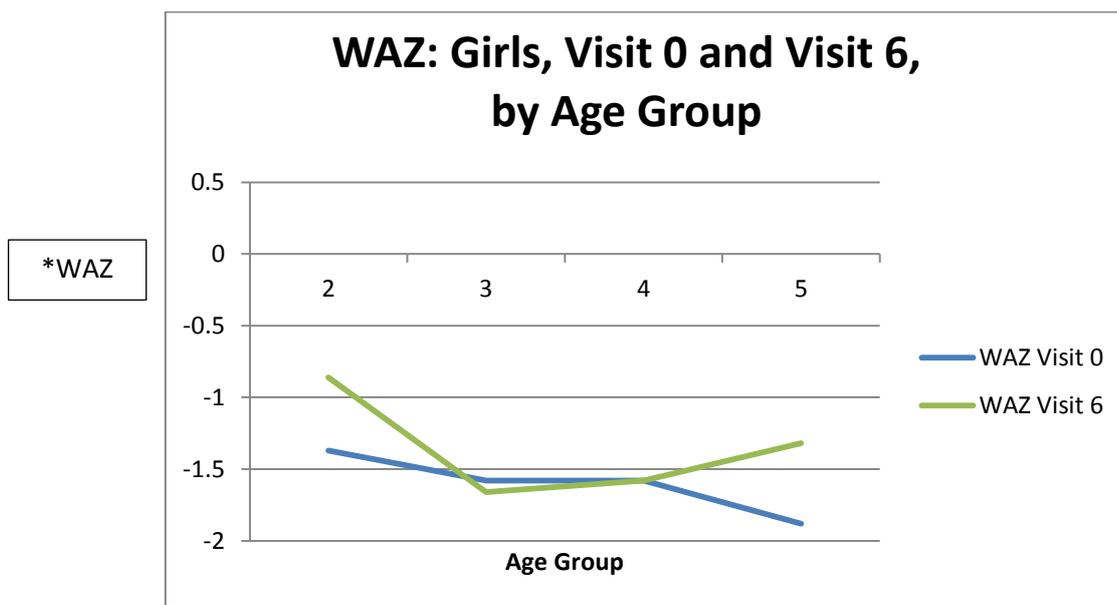


Figure 3.9: Weight-for-age z-score at visit 0 and visit 6 by age-group for girls

3.2.3 Height

Height measurements were performed monthly and plotted according to CDC/NCHS growth references. Data was then analysed by converting percentile data to z-scores. Z-scores for the cohort was categorized according to <-1SD (mildly stunted), <-2SD (moderately stunted) and <-3SD (severely stunted).

The comparison of relative increases in height overtime is presented in Table 3.7. This table shows the absolute height change overtime. The table shows that the greatest increase in height from visit 0 to visit 6 was evident in the 2-year age-group for both boys and girls (4.7cm; 5.5cm respectively) and the 5 year age-group, both boys and girls (4.6cm; 5.8cm respectively). The 2 and 3 year old age-groups experienced marginal increases, but not as substantial as the 2 and 5-year age-groups, for boys (3.4cm;3.9cm respectively) and girls(3.5cm;4.1cm respectively).

Girls for all 4 age-groups experienced the greatest improvement in height over the 6-month period when compared to boys.

Table 3.7: Mean height (cm) of study participants by visit, age and gender *

Visit No.	0		1		2		3		4		5		6		Statistical significance
Gender	M (n=16)	F(n=18)	M (n=14)	F(n=17)	M (n=14)	F(n=16)	M (n=12)	F (n=15)	M(n=11)	F (n=15)	M (n=11)	F (n=14)	M (n=9)	F (n=14)	
2-year*	82.1	81	83.3	81.9	83.8	83.1	84.5	83.9	85.7	85	86.5	85.9	86.8	86.5	p<0.001
3-year*	88.6	88.5	88.5	89.2	88.6	90	90.2	90.8	90	91.2	90.5	91.5	92	92	p<0.239
4-year*	95.5	96.7	97	97.7	96.8	98.9	97.7	99.4	97.7	99.7	98.6	100.4	99.4	100.8	p<0.004
5-year*	100.5	99	101.2	100.2	101.8	102.3	103.4	103	104.1	103.8	105	104.2	105.1	104.8	p<0.011

*each child that was measured for height over the 6-month duration had 6 measurements. Therefore the accumulated total sample size on this table is greater than the sample size (138).

Figures 3.10-3.11 show growth in height at visit 0 and visit 6, by gender.

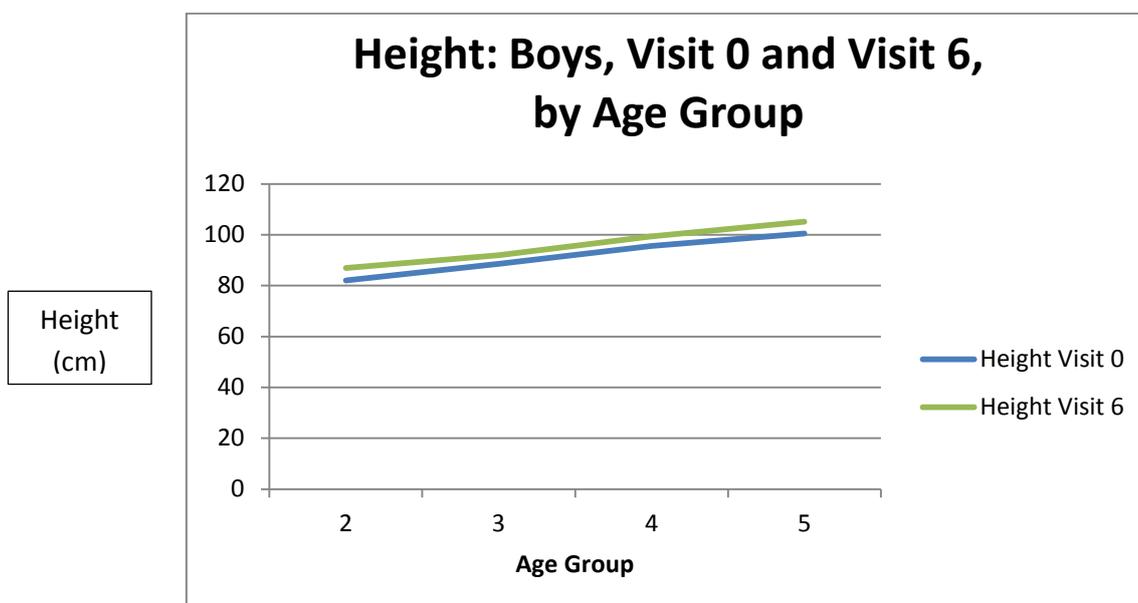


Figure 3.10: Height gain at visit 0 and visit 6 per age-group for boys

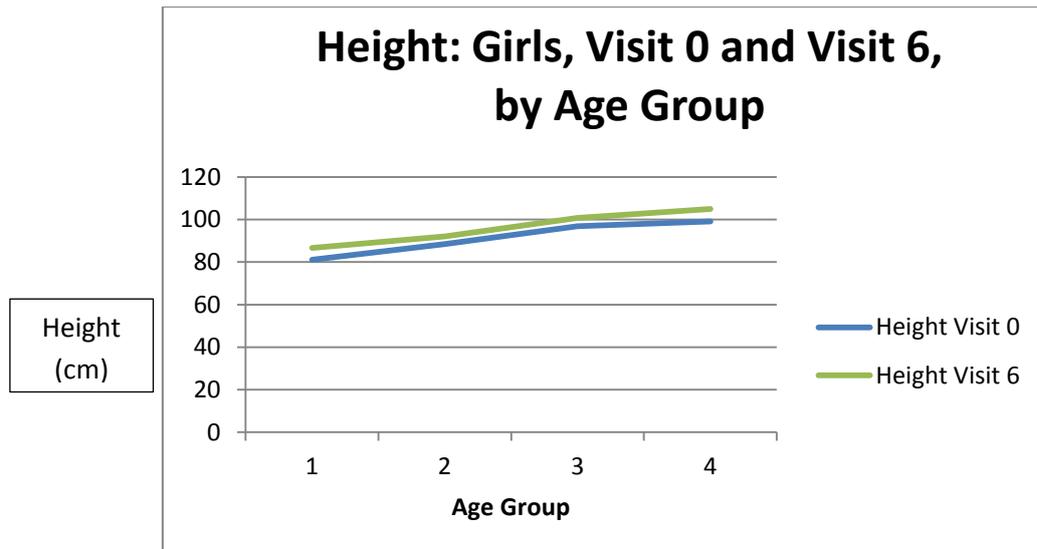


Figure 3.11: Height gain at visit 0 and visit 6 per age-group for girls

Figures 3.12 – 3.14 present the comparison with boxplots per age group height increase over time.

The F-test was > 0.05 (10.57; 3.20; 5.33 respectively), which indicates that all mean values for all the visits were similar for each age-group. For age-group 2, 4, 5-years, a significant improvement in height was seen from visit 0 to visit 6 ($p < 0.0001$; $p = 0.0049$; $p < 0.0001$ respectively).

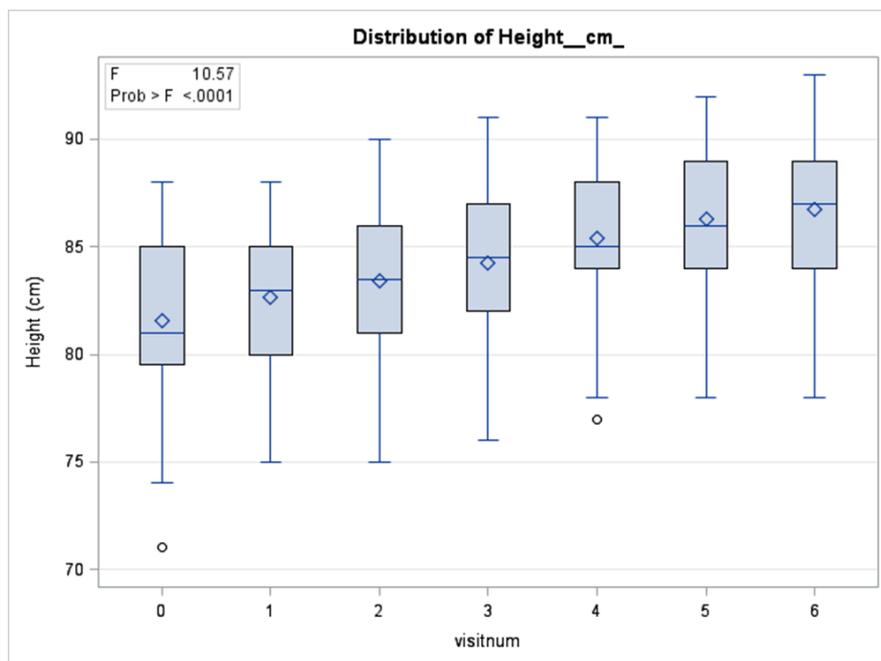


Figure 3.12: Mean absolute height visit 0-6 by age (2-years)

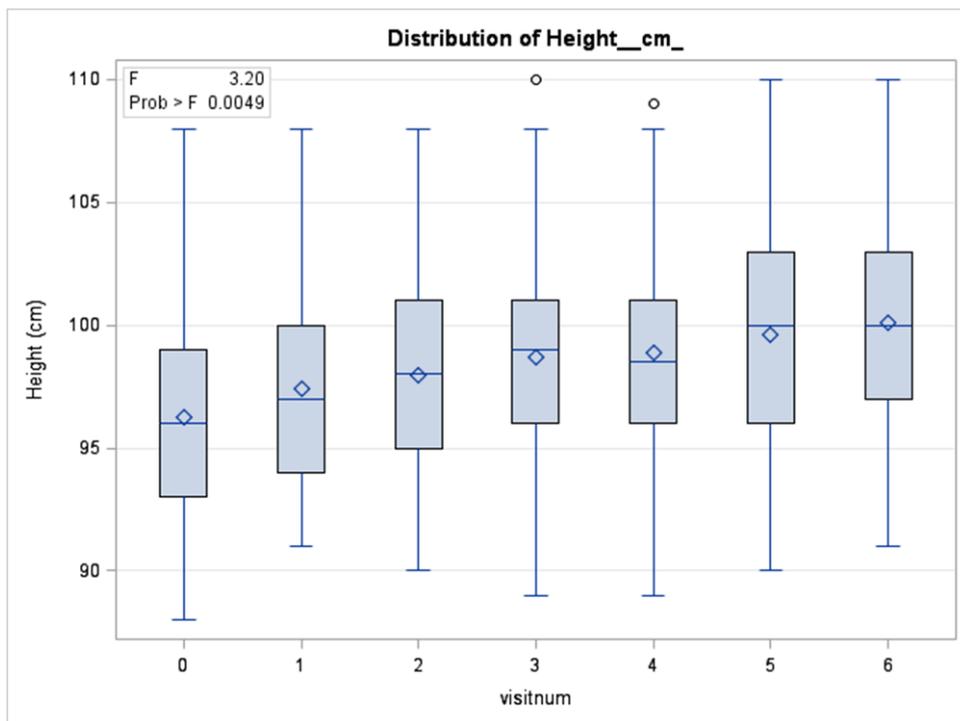


Figure 3.13: Mean absolute height visit 0-6 by age (4-years)

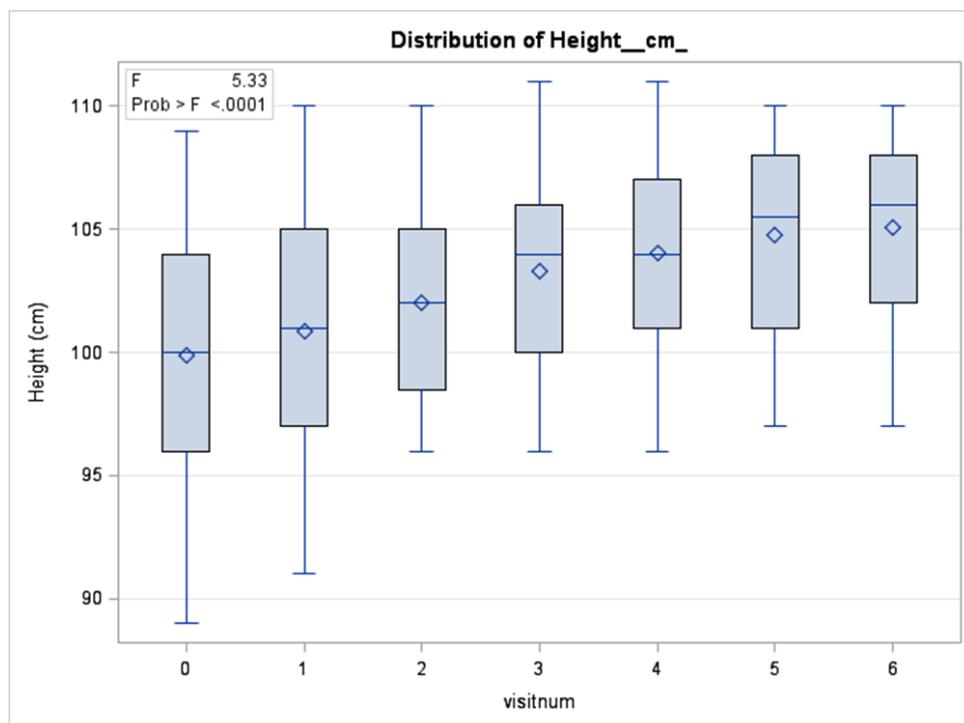


Figure 3.14: Mean absolute height visit 0-6 by age (5-years)

Table 3.8 presents the z-score data at visit 0 and visit 6 (after consumption of supplementation for 6-months). The z-score data was disaggregated by gender and age. For

the 6-months duration of the intervention, the boys within each age-group remained within the moderately stunted category. There was an average incremental change in height overtime for boys of -0.33; this change was not statistically significant. For the girls in the 2, 4 and 5 year age-group, there was an improvement from the moderately stunted category to the mildly stunted category. However, the girls in the 3-year age-group remained moderately stunted for the duration of the intervention.

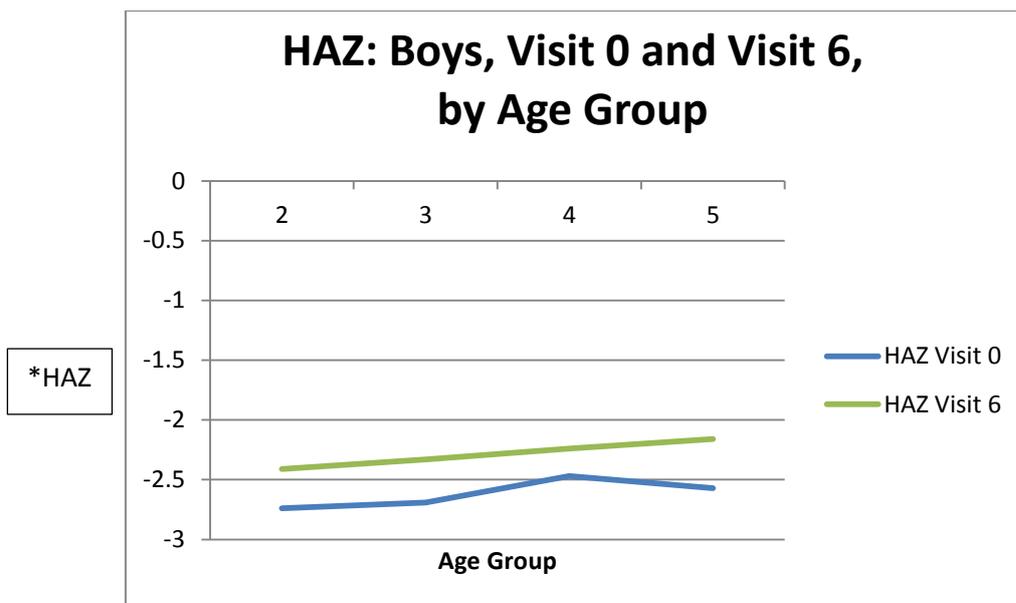
Table 3.8: HAZ of study participants at visit 0 and visit 6

Gender	M* (n=19;10)	F (n*=21;12)	M (n=10;9)	F (n=19;15)	M (n=17;9)	F (21;13)	M (n=18;15)	F (n=13;14)
Age-group	2		3		4		5	
Statistical significance	p<0.112		p<0.998		p<0.888		p<0.133	
HAZ* Visit 0	-2.74	-2.41	-2.69	-2.41	-2.47	2.15	-2.57	-2.54
HAZ Visit 6	-2.41	-1.96	-2.33	-2.31	-2.24	-1.87	-2.16	-1.82
Interpretation	moderately stunted	moderately stunted to mildly stunted	moderately stunted	moderately stunted	moderately stunted	moderate stunted to mildly stunted	moderately stunted	moderately stunted to mildly stunted

* each child that was measured for height over the 6-month duration had 6 measurements. Therefore the accumulated total sample size on this table is greater than the sample size (138).

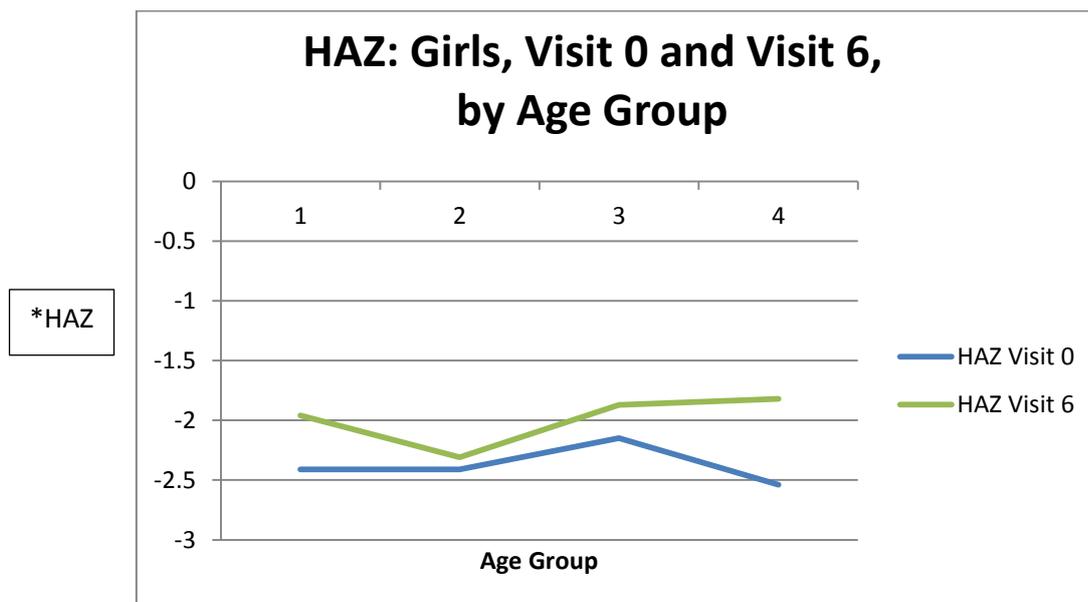
*M: male; n: sample size; F: female; HAZ: weight-for-age Z-score

Figures 3.15 – 3.16 show the gain in height measurements for boys and girls. Both boys and girls experienced improvement in height parameters. The slope of the graph for the boys shows a more linear improvement. After 6-months of consumption all the boys were categorised between the -2 and -2.5 height-for-age z-score. For girls, all age-groups experienced improvement in height measurements; the 2-year and 5-year age-group experienced a more pronounced improvement, as can be seen by the slope of the graph line in Figure 3.16.



*HAZ: Height-for-age z-score

Figure 3.15: Height-for-age z-scores at visit 0 and visit 6 by age-group for boys



*HAZ: Height-for-age z-score

Figure 3.16: Height-for-age z-scores at visit 0 and visit 6 by age-group for girls

Data was further analysed to identify whether height improved in the children who were severely stunted compared to the children who were mildly to moderately stunted. The comparison of height change from visit 0 to visit 6 between the group of children <5th percentile for HFA and the children >5th - 15th percentile for HFA showed no significant difference ($p < 0.070$) (Table 3.9).

When comparing the z-scores, at visit 0, 95% of children had a z-score below the <-1SD, 68% had a z-score <-2SD and 26% had a z-score <-3SD. After 6 months of supplementation, 93% had a z-score <- 1SD, 53% had a z-score <-2SD and 12.5% had a z-score <-3SD. Height change overtime (visit 0 – visit 6) was statistically significant for the <-2SD and the <-3SD ($p<0.007$; $p<0.000$ respectively) categories. Height change overtime was not significant for the <-1SD ($p <0.090$).

Table 3.9: Chi-square Test comparing children >5th -15th percentile HFA <5th percentile HFA between visit 0-6 and z-scores from visit 0 to visit 6

Characteristics	Cohort		Statistical significance
	Visit 0 (n=138)	Visit 6 (n=104)	
Visits			
<5th percentile: HAZ	113 (82%)	77 (74%)	$p<0.070$
>5th percentile: HAZ	24 (17%)	27 (26%)	
Z-score: HAZ			
<-1SD	132 (95%)	97 (93%)	$p<0.090$
<-2SD	95 (68%)	56 (53%)	$p<0.007$
<-3SD	36 (26%)	13 (12.5%)	$p<0.000$

3.3 ANALYSIS OF OBJECTIVE 2

To determine the effect of a complete nutritional supplement given in addition to the daily food intake on the levels of selected immune markers (C-reactive protein, faecal calprotectin and faecal IgA) in a cohort of underweight HIV-positive children aged 24-72 months.

The reference range used for CRP in this study was $\leq 10\text{mg/l}$. An elevated median CRP $>10\text{mg/l}$ indicates the presence of inflammation. There were only 21 participants who had a CRP sample at visit 0 and again at visit 6. Insufficient blood volume was collected in the majority of cases. The main study required a blood volume of 10mL for blood tests mainly to assess safety aspects, including blood formula and cell numbers. If a low volume of blood was collected from the child, blood tests were placed in order of priority based on the main study objectives. CRP was a secondary objective of the main study and therefore was not listed as a main priority blood test. An insufficient volume was collected in a case where a participant became distressed due to fear of phlebotomy or a difficult peripheral venous access.

Table 3.10 presents the median of CRP values for participants who had a CRP sample at visit 0 and visit 6. The CRP median result at visit 0 was 18.4mg/l , and, after 6-months of supplementation the CRP median result decreased to 3.90mg/l which is a substantial decrease in inflammation. The median was the preferred approach of presentation, because

the data distribution was skewed, and therefore the mean could be misleading. The minimum range for the CRP samples analysed of the 21 participants was equivalent to the reference range and after 6-months of supplementation, the minimum was calculated at 0.40mg/l and the maximum 52.4mg/l.

Table 3.10: Median CRP (mg/l) results for the cohort at visit 0 and visit 6

Measure	Visit 0	Visit 6
CRP (n)	21*	21*
Male	14	14
Female	7	7
Minimum range	10.20mg/l	0.40mg/l
Maximum range	236mg/l	52.40mg/l
Median	18.40mg/l	3.90mg/l

**Number of children that had a blood sample analysed at visit 0 and visit 6*

The box plot (Figure: 3.17) presents the change in CRP values over the 6-months. Statistical significance was calculated using the Related-Samples Wilcoxon Signed Rank Test. The change in CRP levels from visit 0 – visit 6 showed a significant improvement (decrease) in CRP levels ($p < 0.002$).

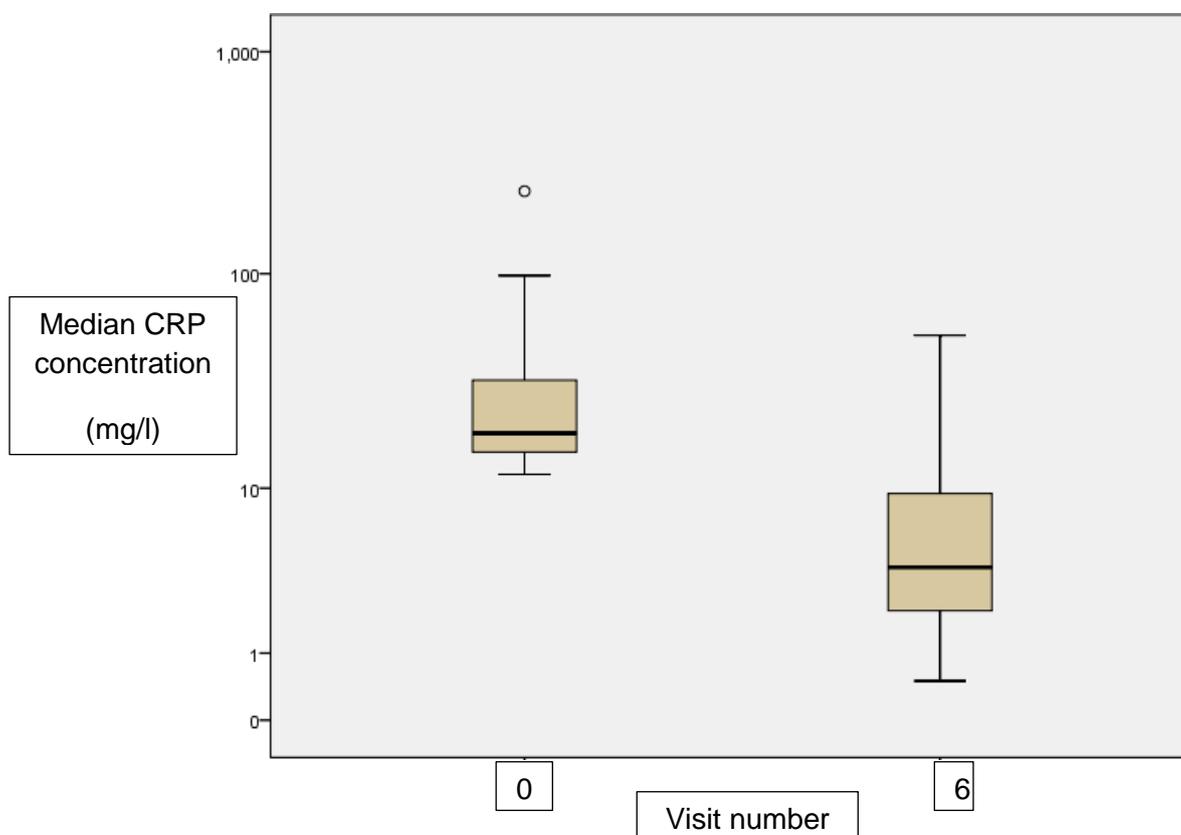


Figure 3.17: Median CRP results of study participants at visit 0 to 6

The data was further analysed to identify whether CRP levels decreased differentially in the group of children <5th percentile for weight-for-age compared to the group of children >5th - 15th percentile for weight-for-age. For both groups, CRP levels were above the reference range at visit 0 (11mg/l; 14.9mg/l respectively). By visit 1 an improvement was already identified as both groups' mean CRP dropped (7.8mg/l; 9.3mg/l respectively) to within the reference range of ≤10mg/l. By visit 6, the CRP levels had improved for both groups (4.2mg/l; 6.4mg/l respectively). Although the children with a WFA <5th percentile had a mean CRP of 11mg/l, which is close to the reference range, their CRP results decreased rapidly to within the normal range. Although improvement was seen, by the reduction of CRP levels, the change in CRP was not statistically significant for each group at the three visits ($p < 0.553$; $p < 0.738$; $p < 0.326$ respectively).

Figure 3.18 shows the improvement in CRP levels to normal ranges for both the children <5th percentile for WFA and those >5th - 15th percentile for WFA. The children >5th - 15th percentile had a higher visit 0 CRP level (14.9mg/l) compared to the children <5th percentile (11mg/ml). However, both groups at visit 6, had CRP levels within the normal range.

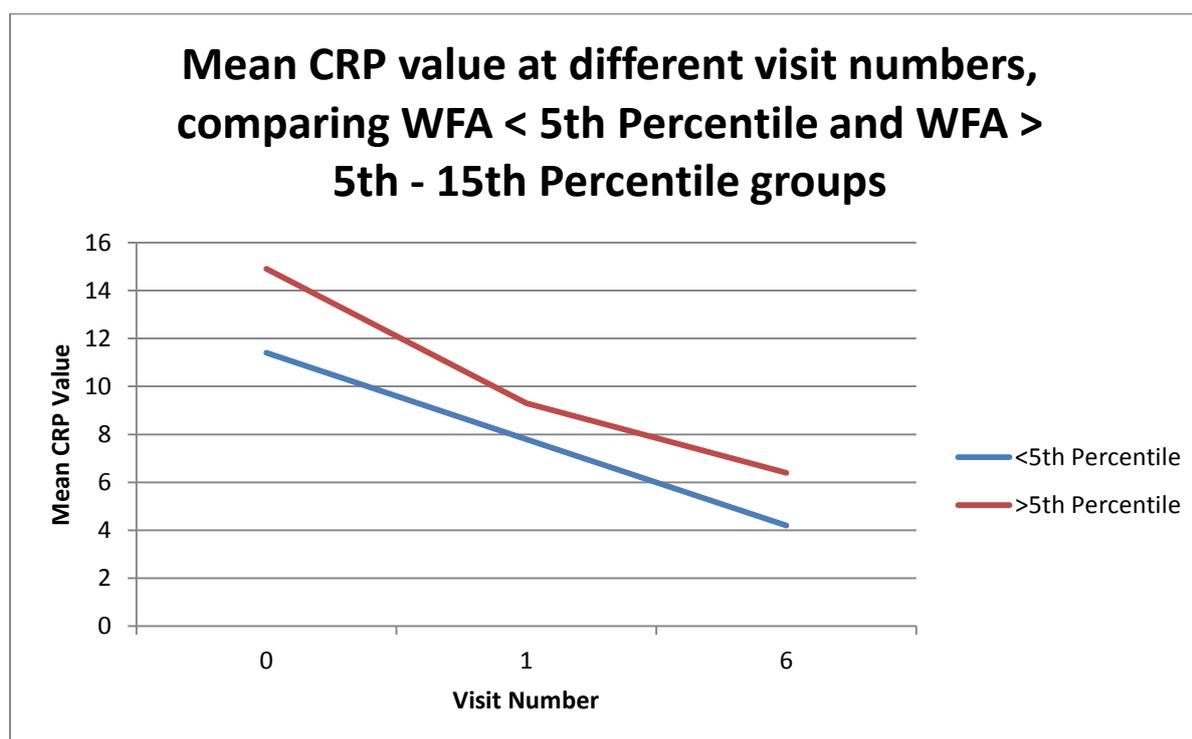


Figure 3.18: Comparison of mean CRP values between children <5th percentile for WFA and children >5th - 15th percentile for WFA

CRP levels were compared between the group of children <5th percentile for HFA compared to the group of children >5th - 15th percentile for HFA. For both groups, CRP levels were

above the reference range (13.2mg/l; 14.5mg/l respectively). By month 6, the CRP levels had improved, such that the mean CRP levels were within the normal range of ≤ 10 mg/l for both groups (5.7mg/l; 5.9mg/l respectively). The change overtime in CRP levels was similar for both groups. A change in CRP levels was seen in both groups, however, the change was not statistically significant ($p < 0.868$; $p < 0.611$; $p < 0.940$ respectively).

Figure 3.19 shows the mean CRP level improving to within the normal ranges for both groups of children. Again it can be seen that the children with a $>5^{\text{th}}$ – 15^{th} percentile for HFA started with an increased CRP level at visit 0 (14.5mg/l), but improved by month 6 to within the normal range (5.9mg/l).

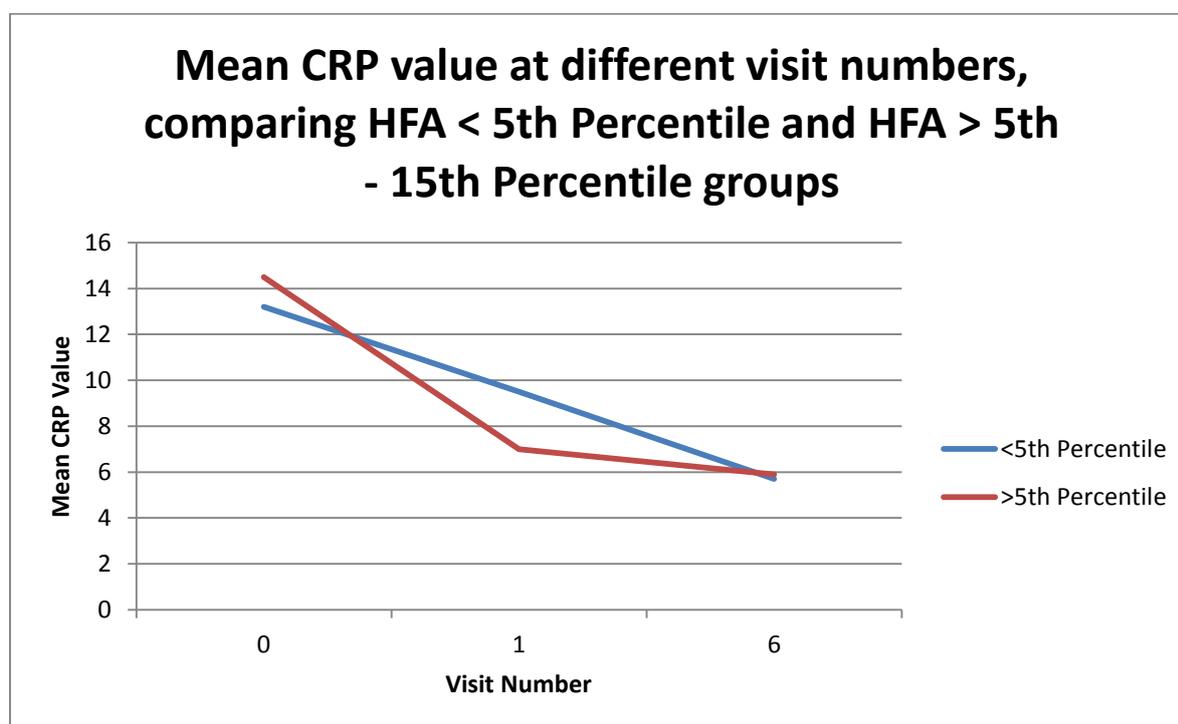


Figure 3.19: Comparison of mean CRP values between children <5th percentile for HFA and children >5th – 15th percentile for HFA

3.3.1 Calprotectin

The reference range used in this study for Calprotectin = ≤ 50 ug/g. An elevated median calprotectin value of >50 ug/g indicates the presence of intestinal inflammation. Table 3.11 presents the calprotectin data. Only 22 children had a sample for analysis at visit 0 and visit 6. This was a small sample size, due to the difficulty in collecting the required volume of faecal sample. In most cases a low volume of faecal sample was collected, this meant the faecal tests were placed in order of priority based on the main study objectives. Calprotectin was a secondary objective of the main study and therefore was not listed as a main priority faecal test.

The median value at visit 0 was 127ug/g of feces. At visit 6 the median was reduced to 31.8ug/g which is within the normal ranges, and is indicative of an improvement or reduction in inflammation. The median was the preferred approach of presentation, because the data distribution was skewed, and therefore the mean could be misleading.

Table 3.11: Median calprotectin results of the cohort at visit 0 and visit 6

Measure	Visit 0	Visit 6
Calprotectin (N)	22*	22*
Male	8	8
Female	14	14
Minimum range	53ug/g	9.80ug/g
Maximum range	381ug/g	357.96ug/g
Median	127.38ug/g	32ug/g

**Number of children that had a blood sample analysed at visit 0 and visit 6*

The box plot (Figure 3.20) below presents the change in calprotectin values over the 6-months. The statistical significance was calculated using the Related-Samples Wilcoxon Signed Rank Test. The change in calprotectin levels from visit 0 to visit 6 showed a significant improvement (reduction) in calprotectin levels ($p < 0.005$)

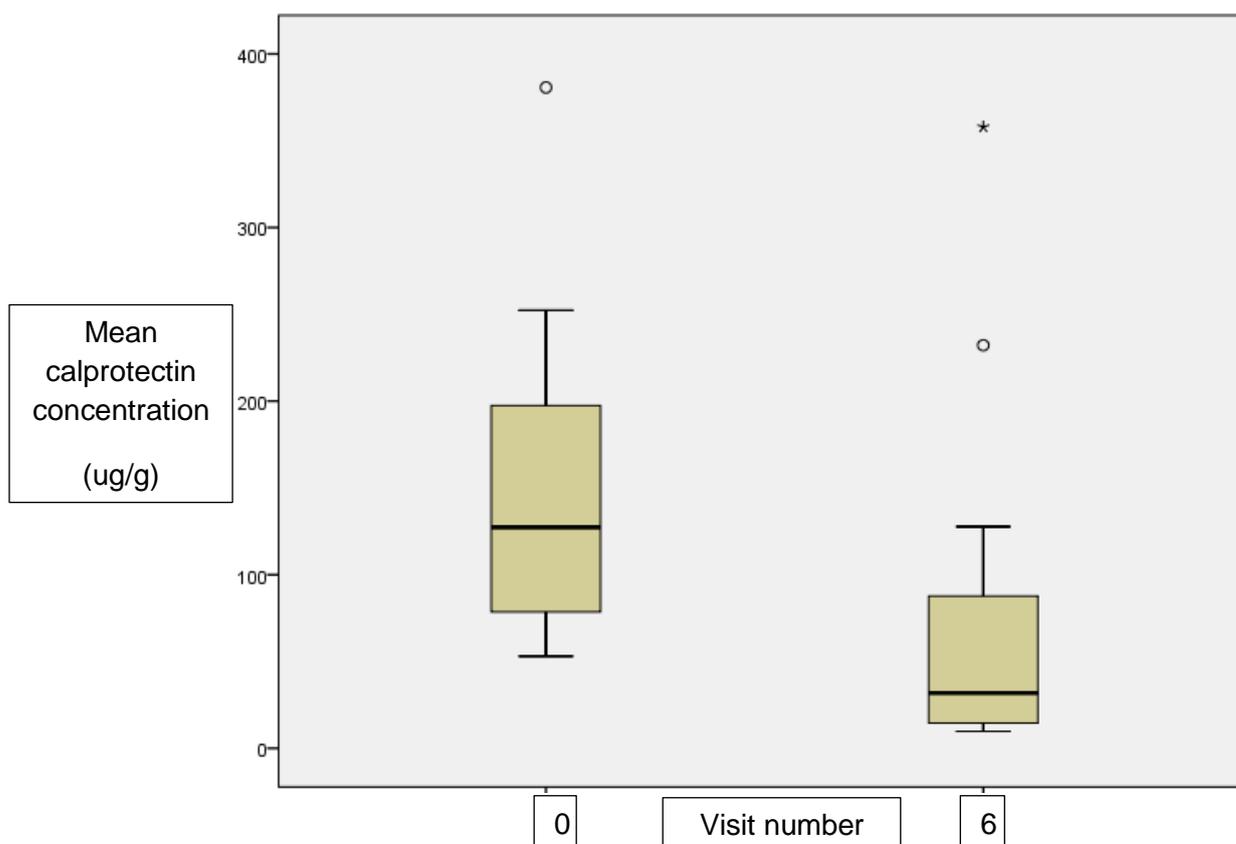


Figure 3.20: Median calprotectin results of study participants at visit 0 – 6

A comparison of calprotectin levels were carried out between the group of children <5th percentile for WFA compared to the group of children >5th – 15th percentile for WFA. For the group of children below the 5th percentile for WFA, the mean calprotectin level was above the normal range (58.3ug/g), at month 1 (85.9ug/g), an increase is noticed and by month 6, the calprotectin level is reduced (61.6ug/g), but not within the normal range. In comparison, the group of children with a WFA between the 5th – 15th percentile had an increased calprotectin level at visit 0 (81.2ug/g), by month 1 the calprotectin was still raised (86.8ug/g), but at month 6 the calprotectin result for the group was reduced to close to the reference range (54.2 ug/g). Calprotectin levels were reduced, however, the change in calprotectin levels over time was not significant at the three visits (p<0.103;p<0.959;p<0.658 respectively).

Figure 3.21 shows the comparison of calprotectin values from visit 0 to visit 6 between the >5th – 15th percentile for WFA and the <5th percentile for WFA, graphically.

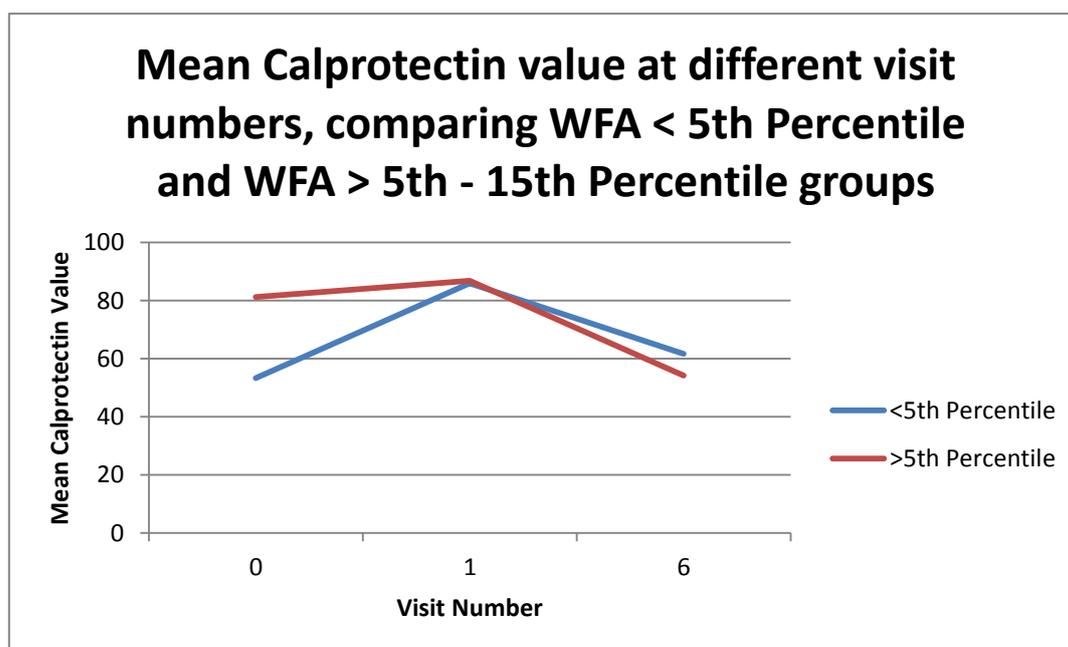


Figure 3.21: Comparison of mean calprotectin values between children <5th percentile for WFA and children >5th – 15th percentile for WFA

A comparison of calprotectin levels were carried out for the group of children <5th percentile for HFA compared to the group of children >5th – 15th percentile for HFA. For the group of children below the 5th percentile for HFA, the calprotectin level was above the normal range (71.2ug/g), at month 1, a substantial increase is noticed and by month 6, the calprotectin level is reduced (59ug/g), but not within the normal range. In comparison, the group of children with a HFA between the 5th – 15th percentile had an increased calprotectin level at

visit 0 (58.3ug/g), by month 6 the calprotectin level for the group was within the reference range (48.5ug/g). Although the, calprotectin levels decreased, this change in calprotectin level was not statistically significant across the two groups ($p < 0.572$; $p < 0.939$; $p < 0.548$ respectively).

Figure 3.22 provides a graphical representation of the change in calprotectin level for children $< 5^{\text{th}}$ percentile and children between the $5^{\text{th}} - 15^{\text{th}}$ percentile for HFA.

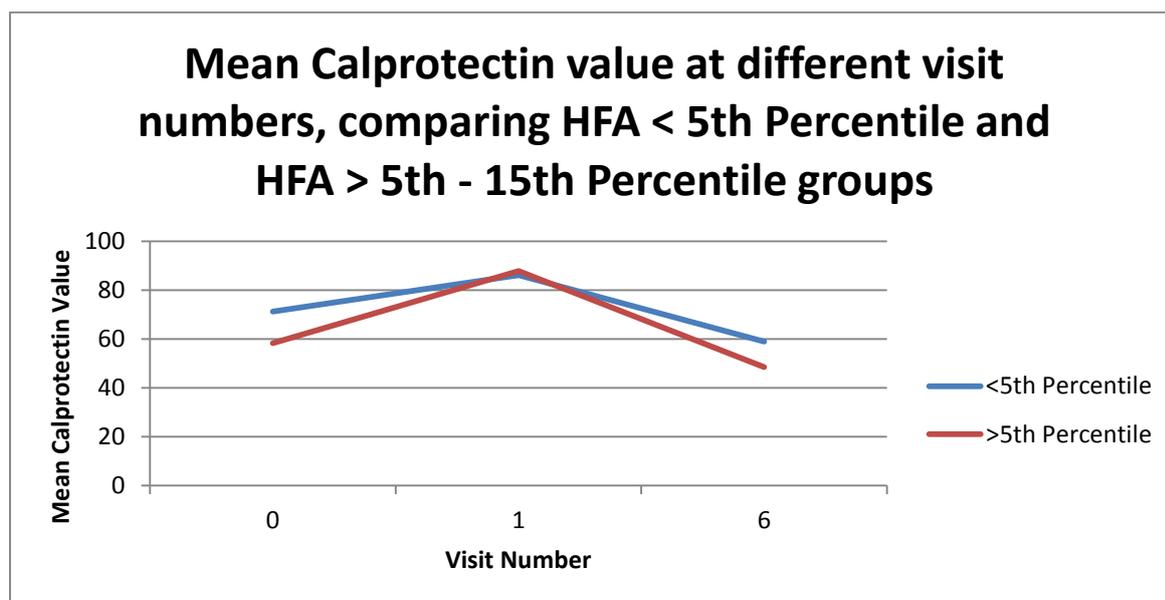


Figure 3.22: Comparison of mean Calprotectin values between children $< 5^{\text{th}}$ percentile for HFA and children $> 5^{\text{th}} - 15^{\text{th}}$ percentile for HFA

3.3.2 Immunoglobulin A

The reference range used for IgA values in children is between 510ug-2040ug/ml. A value below 510ug/ml may indicate a reduced activity of the intestinal immune system, and may be associated with an autoimmune deficiency⁽³²⁾. A value > 2040 ug/ml likely indicates an infectious episode⁽³²⁾.

Table 3.12 presents the IgA data. Only 17 children had a sample for analysis at visit 0 and at visit 6. This was a small sample size, due to the difficulty in collecting the required volume of sample. If a low volume of faecal sample was collected, the faecal tests were placed in order of priority based on the main study objectives. IgA was a secondary objective of the main study and therefore was not listed as a main priority faecal test. The median value at visit 0 was 6623ug/ml. At visit 6 the median was reduced to 3534ug/ml which falls outside of the reference range. The median was the preferred approach of presentation as the measurement for central tendency, because the data distribution was skewed, and therefore the mean could be misleading. The minimum range at visit 0 was > 2040 ug/ml for IgA, at visit

6 the minimum range was as low as 363ug/ml and the maximum range was 6810ug/ml. The median at both visit 0 and visit 6 was >2040ug/ml.

Table 3.12: Median IgA results of study participants from visit 0 to visit 6

Measure	Visit 0*	Visit 6*
IgA Samples analysed	17	17
Male	2	2
Female	15	15
Minimum range	2057ug/ml	363ug/ml
Maximum range	22212ug/ml	6810ug/ml
Median	6624ug/ml	3534ug/ml

*Number of children that had a blood sample analysed at visit 0 and visit 6

The box plot (figure 3.23) below presents the change in IgA values over the 6-months period. The statistical significance was calculated using the Related-Samples Wilcoxon Signed Rank Test. The change in IgA levels from visit 0 to visit 6 showed a significant improvement (reduction) in IgA levels according to the Wilcoxon Signed Rank test ($p < 0.003$). The ranges were still above the maximum range which is associated with the HIV infection resulting in bacterial overgrowth in the GIT.

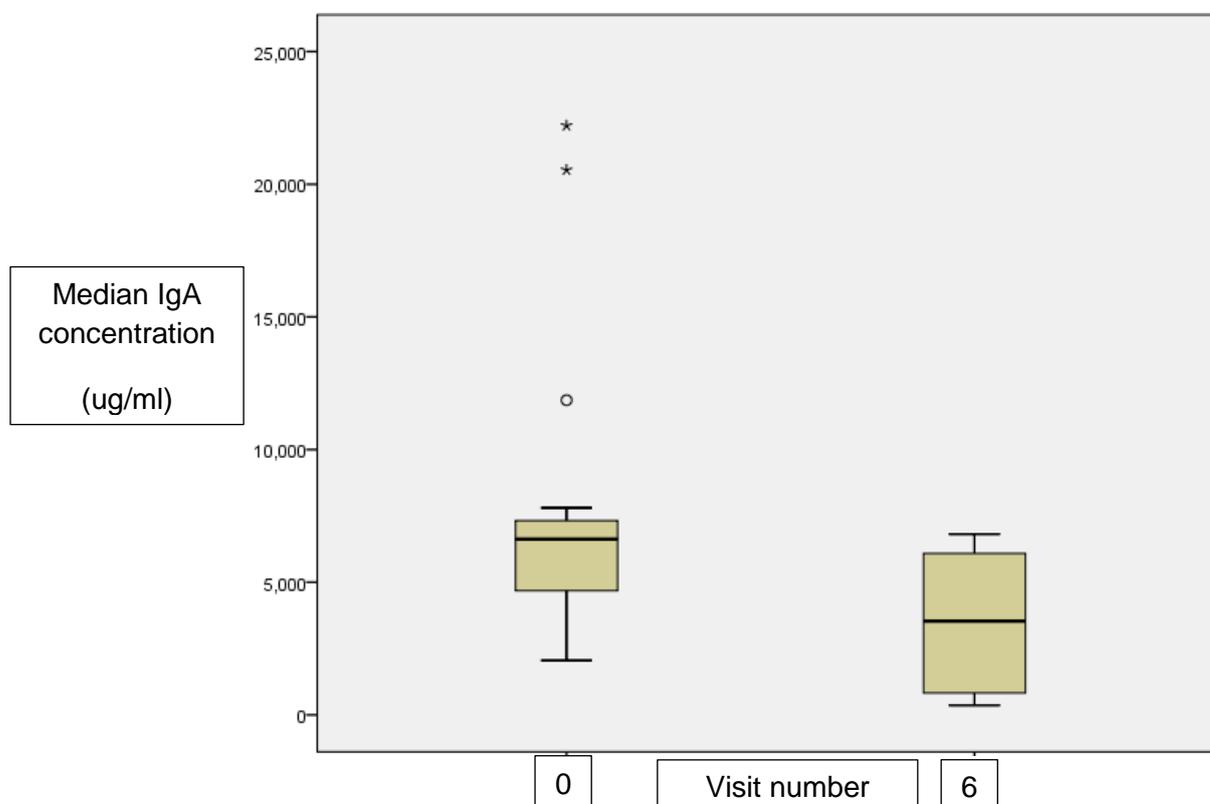


Figure 3.23: Median IgA results of study participants at visit 0 to visit 6

A comparison of IgA mean results were carried out for the group of children <5th percentile for WFA compared to the group of children >5th – 15th percentile for WFA. Although IgA mean results for both groups remained elevated, the data shows that the levels of IgA decreased in the direction of the reference range. The change in IgA levels overtime was not statistically significant for the groups ($p < 0.660$; $p < 0.203$; $p < 0.758$ respectively). Figure 3.24 provides a graphical representation of the data. The line graph provides a clear visual as to the trend of IgA levels for each group.

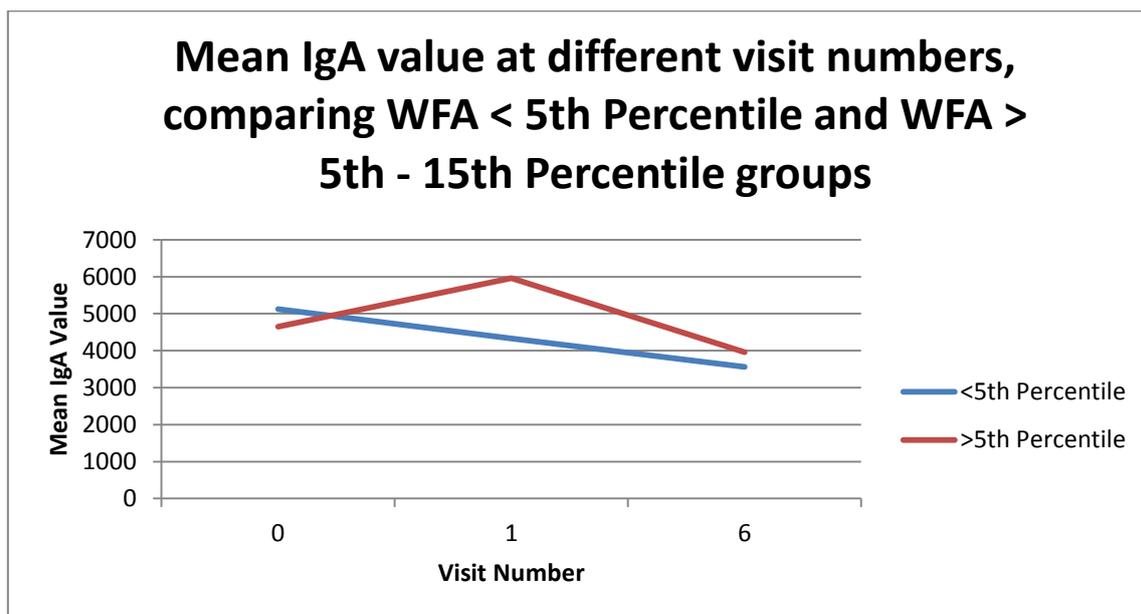


Figure 3.24: Comparison of mean IgA values between children <5th percentile for WFA and children >5th - 15th percentile for WFA

A similar picture is noted for the <5th percentile and >5th - 15th percentile for HFA groups. Both groups had elevated IgA levels at the onset of the study (4606ug/ml; 5680ug/ml respectively) and by month 1 a reduction in IgA levels was seen (5516ug/ml; 5036ug/ml respectively). However, at month 6 the >5th – 15th percentile HFA group was more elevated compared to the group <5th percentile for HFA. For both groups the change in IgA levels was not significantly different between the two ($p < 0.391$; $p < 0.739$; $p < 0.585$). Figure 3.25 provides a visual representation of the data.

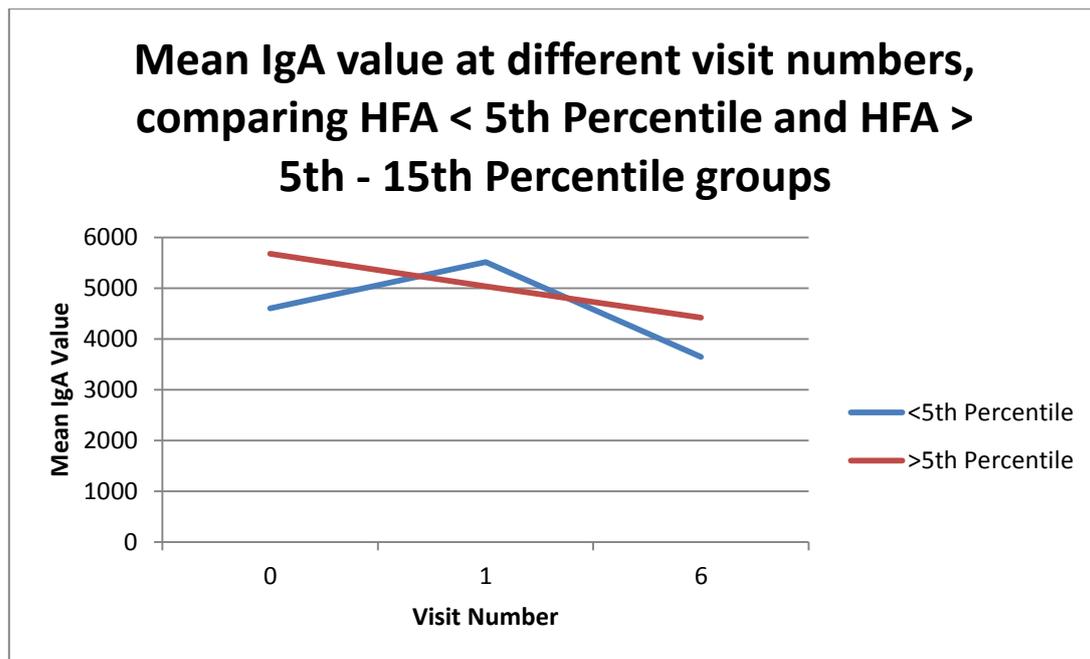


Figure 3.25: Comparison of mean IgA values between children <5th percentile for HFA and children >5th – 15th percentile for HFA

3.4 ANALYSIS OF OBJECTIVE 3

To explore how prebiotics, probiotics and DHA influence growth (height and weight) and immune markers of a cohort of underweight HIV positive children aged 24-72 months.

3.4.1 Weight

To determine whether the supplement given to group B (i.e. receiving the basic supplement containing additional (prebiotics, probiotics and DHA) nutrients and given in addition to the daily food intake) had a greater impact on Table 3.13 shows the z-score breakdown for group A and B. If there was a significant improvement in weight gain from visit 0 to visit 6, a corresponding p-value was provided.

The z-score analysis comparing the childrens weight gain in group A compared to group B, found no significant difference across the two groups (visit 0= $p < 0.641$; $p < 0.438$; $p < 0.999$ respectively) (visit 6= $p < 0.140$; $p < 0.999$; $p < 0.315$). When you consider Table 3.19, 82% of children were below the $< -1SD$ for group A and for group B 85%. For children in group A, only 23% were below the $< 2SD$ and 28% in group B. In group A, 2.9% of the children were below the $< -3SD$ and in group B 2.9%. After 6-months of supplement, 61% of the children from group A still remained below the $< -1SD$ and 75% from group B. For group A 13% of children were below the $< -2SD$ and 13% from group B. For $< -3SD$ and no children from group A, but from group B, 1.9% still remained below the $< -3SD$.

Table 3.13: Chi-square test comparing WAZ between group A and group B at visit 0 and 6

Group Visit	Visit 0		Significance	Visit 6		Significance
	Group A (%)	Group B (%)	p value	Group A (%)	Group B (%)	p value
Visit 0: <-1SD	82	85	0.6419	61	75	0.1403
Visit 0: <-2SD	23	28	0.4381	13	13	0.999
Visit 0: <-3SD	2.9	2.9	0.999	0	1.9	0.315

The data was analysed further to compare the effects of the individual nutritional supplement on absolute weight. Figure 3.26 shows the difference in growth for group A and group B by visit number and for girls. For all girls at each visit, there was no significant difference between the two groups in weight gain at the visit 0 – visit 6 ($p < 0.558$; $p < 0.848$; $p < 0.526$; $p < 0.701$; $p < 0.862$; $p < 0.579$; $p < 0.929$ respectively). Therefore the supplements had the same effect on weight gain. A similar non-significant effect was shown for boys (Figure 3.27)

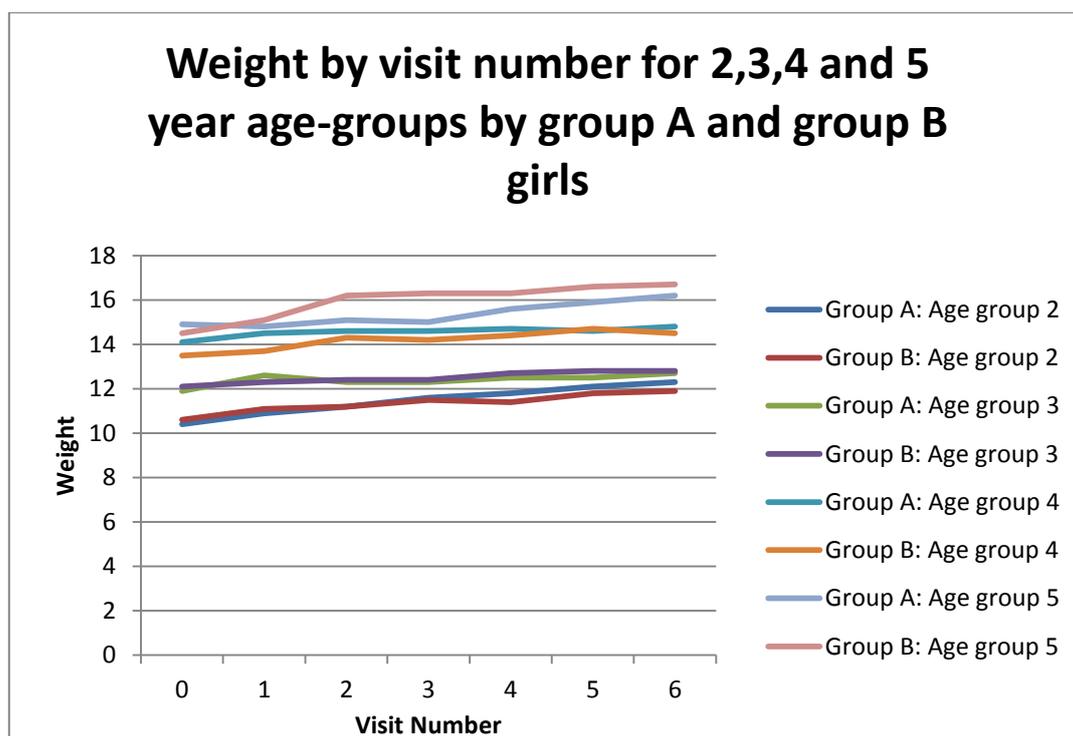


Figure 3.26: Comparison of group A and B weights at visit 0 to 6 by age-group (girls)

When comparing the boy’s weight gain across visits, a similar trend was seen. The weight gain for the boys across visits was not statistically significant ($p < 0.660$; $p < 0.457$; $p < 0.336$;

p<0.080; p<0.276; p<0.231; p<0.187 respectively). Therefore the supplements had the same effect on weight gain for the boys at each visit (Figure 3.27).

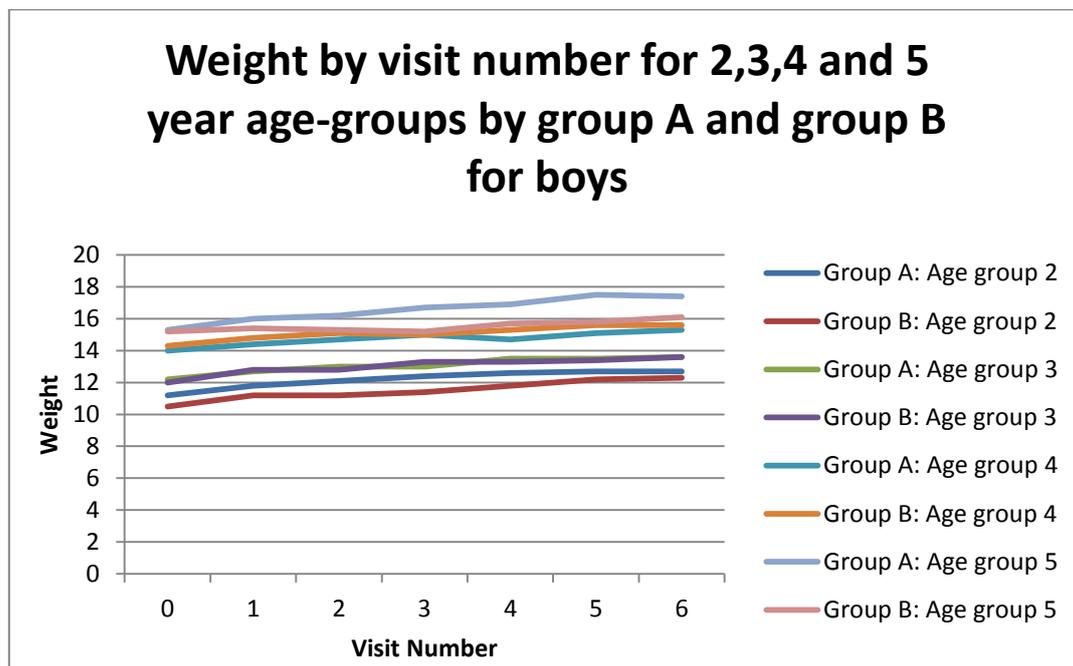


Figure 3.27: Comparison of group A and B weights at visit 0 to 6 by age-group (boys)

3.4.2 Height

To determine whether the supplement given to group B had a greater impact on growth when compared to group A, a comparison of data between group A and group B was carried out.

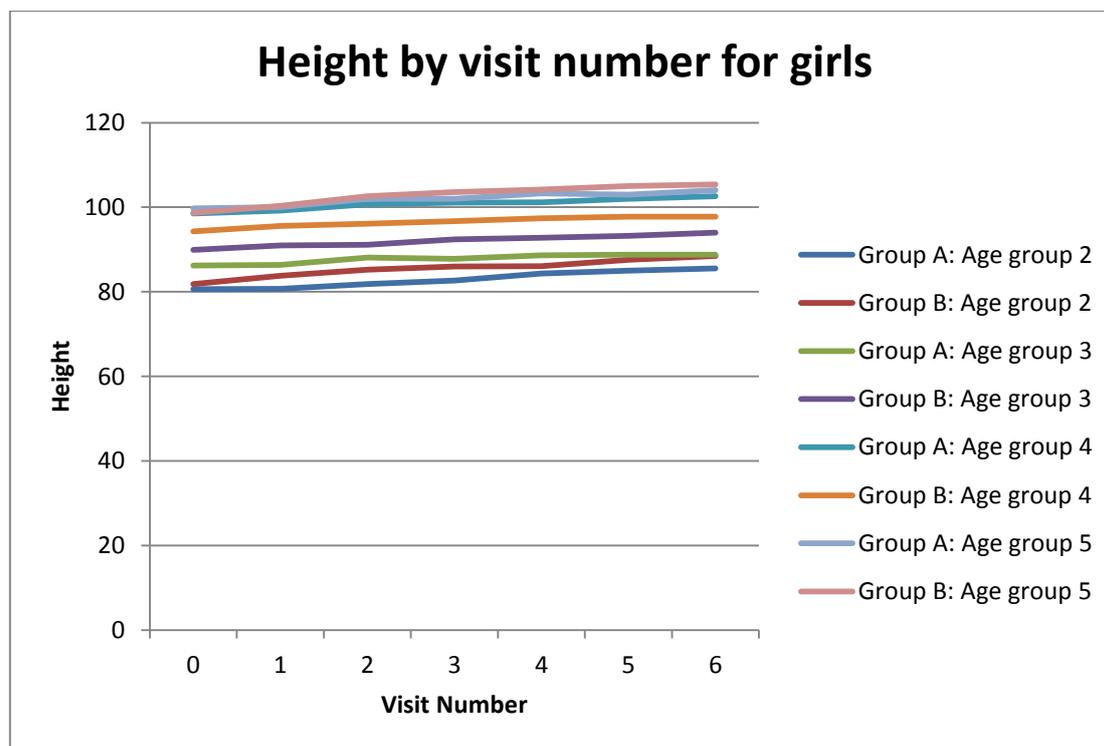
For group A, 95% of children were below the -1SD (mildly stunted) and in group B, also 95% of children. In group A, 72% of the children were below the <-2SD (moderately stunted) compared to group B who had 65% of children <-2SD. For <-3SD (severely stunted) 26% of the children from group A and 26% of children from group B. None of these differences were significant.

At visit 6, 96% of children from group A were <-1SD and 90% of children from group B. For group A, only 55% were below <-2SD and for group B, 51%. 19% of children from group A were below the <-3SD and only 5.7% of children from group B. The change in height was statistically significant for the <-3SD children (p<0.037). Group B children experienced a substantial change in height gain compared to group A (Table 3.14).

Table 3.14: Chi-square test comparing HAZ between group A and group B

Group Visit	Visit 0		Significance	Visit 6		Significance
	Group A (%)	Group B (%)	p value	Group A (%)	Group B (%)	p value
Visit 0: <-1SD	95	95	0.999	96	90	0.2404
Visit 0: <-2SD	72	65	0.3581	55	51	0.694
Visit 0: <-3SD	26	26	0.999	19	5.7	0.0379

The data was analysed further to compare the effects of the individual nutritional supplement on absolute height. Figure 3.28 shows the difference in growth for group A and group B by visit number and for girls. For all girls at each visit, there was no significant difference between the two groups in height gain at the visit 0 – visit 6 ($p < 0.454$; $p < 0.287$; $p < 0.363$; $p < 0.376$; $p < 0.572$; $p < 0.388$; respectively). Therefore the supplements had the same effect on height gain.

**Figure 3.28: Comparison of height gain by age-group, visit number and group (girls)**

When comparing the boy's height gain across visits, a similar trend was seen. The height gain for the boys across visits was not statistically significant ($p < 0.833$; $p < 0.800$; $p < 0.948$; $p < 0.587$; $p < 0.842$; $p < 0.636$; respectively). Therefore the supplements had the same effect on weight gain for the boys (Figure 3.29).

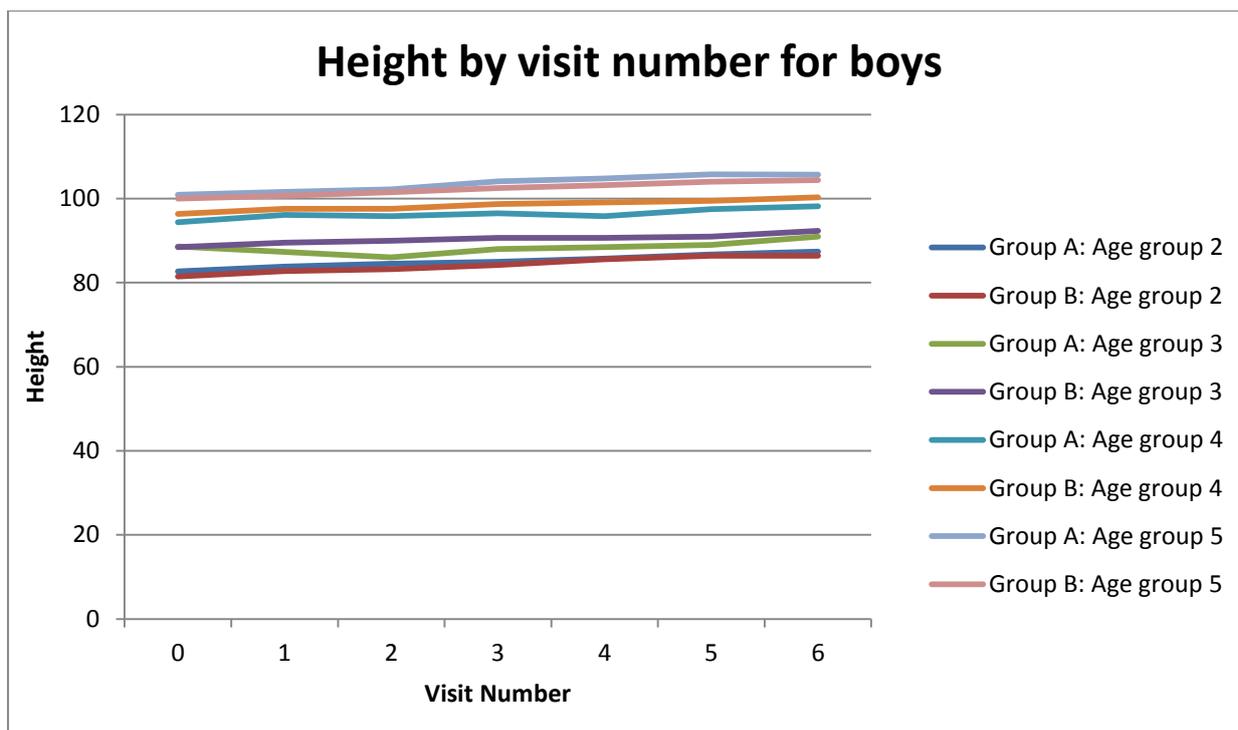


Figure 3.29: Comparison of height gain by age-group, visit number and group (boys)

3.4.3 C-reactive protein

When comparing the effect of the two supplements on the groups of children with a WFA below the 5th percentile, the levels of CRP were decreased to within the normal ranges (6.4mg/l; 2.2mg/l respectively) in both groups at the 6 months visit (Table 3.15).

A comparison between the two groups showed no significant difference between the two groups ($p < 0.690$; $p < 0.137$ respectively) at visit 0 and 1. However, at visit 6, a statistically significant difference is shown ($p < 0.038$), indicating group A had substantial improvement in CRP levels over 6-months. Clinically though, both groups had CRP levels within the normal range.

Table 3.15: Group A and B (WFA) CRP results by percentiles and visit

Visit No.	N	<5th Group A	N	<5th Group B	p-value	N	>5th Group A	N	>5th Group B	p-value
0	20	12.5mg/l	30	10.7mg/l	0.711	35	13.2mg/l	29	17.1mg/l	0.690
1	15	9.8mg/l	18	6.2mg/l	0.413	35	4.7mg/l	38	13.6mg/l	0.137
6	12	6.4mg/l	13	2.2mg/l	0.073	34	3.4mg/l	30	9.3mg/l	0.038

When comparing the effect of the two supplements on the groups of children with a HFA below the 5th percentile, both groups CRP levels decreased to within the normal ranges and none of the differences were statistically significant (Table 3.16).

Table 3.16: Group A and B (HFA) CRP results by percentiles and visit

Visit No.	N	<5th Group A	N	<5th Group B	p-value	N	>5 th Group A	N	>5 th Group B	p-value
0	45	15.3mg/l	11.2	47mg/l	0.444	9	1.8mg/l	24	12mg/l	0.336
1	38	6.5mg/l	12.3	41mg/l	0.295	12	5.3mg/l	8.3	15mg/l	0.584
6	36	4.7mg/l	6.9	31mg/l	0.360	10	3.8mg/l	7.6	12mg/l	0.273

3.4.4 Calprotectin

All percentile groups had elevated calprotectin values at visit 0 (except group B <5th percentile which had normal values; Figure 3.30; $p < 0.103$). The trend in calprotectin levels over time was to increase at visit 1 (except group A >5th -15th percentile) followed by a return to near normal values at visit 6 (except for group A < 5th percentile which remained above the upper limit of normal). None of these differences were significant ($p < 0.341$; $p < 0.275$; $p < 0.851$).

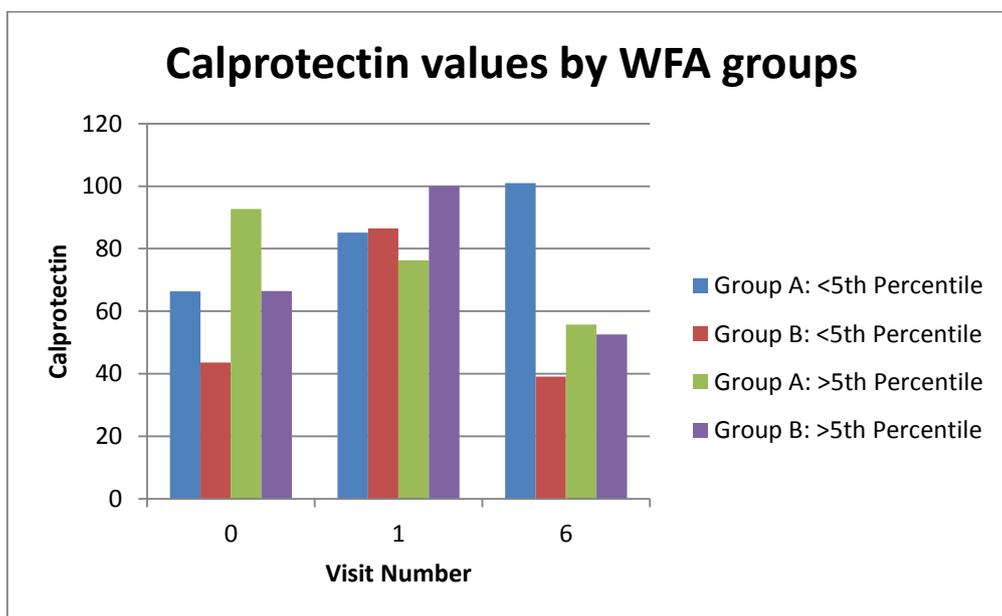


Figure 3.30: Comparison of calprotectin values by group and percentile for WFA

A similar trend was seen with HFA category. Calprotectin levels were increased at month 1 followed by a return to normal values at visit 6. Only the children <5th percentile receiving supplement A, did not have calprotectin levels within the normal range by month 6. None of these differences were significant ($p < 0.479$; $p < 0.616$; 0.755 respectively). The significance of

the children in group A <5th percentile was due to the increased calprotectin levels at visit 0 ($p < 0.040$). Figure 3.31 provides a visual comparison between group A and B, by percentile HFA.

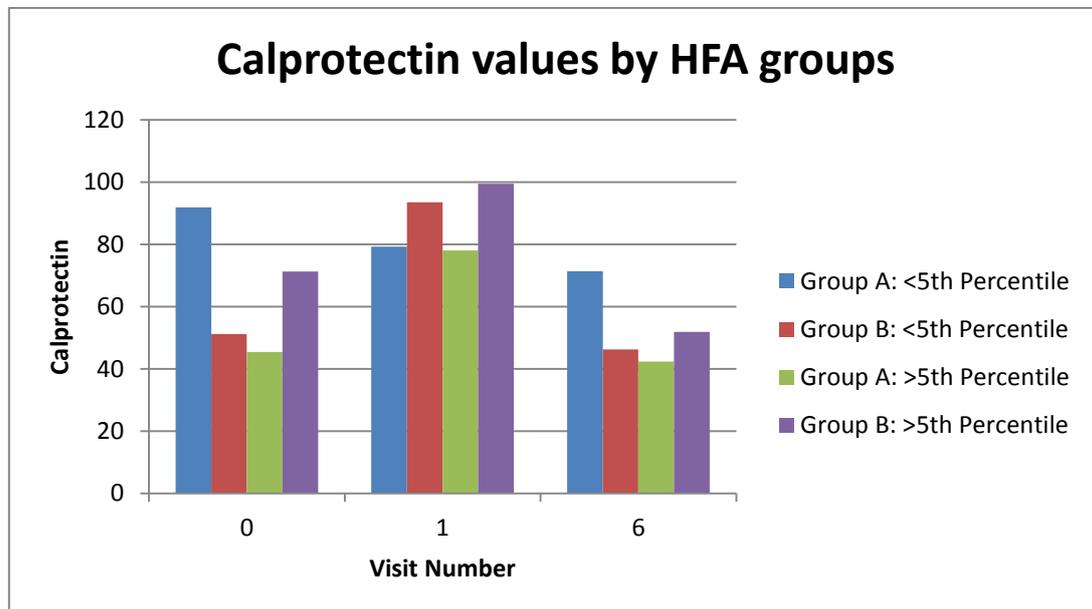


Figure 3.31: Comparison of calprotectin values by group and percentile for HFA

3.4.5 Immunoglobulin A

The >5th – 15th percentile group of children for WFA and HFA did not have any statistical significant difference between the two groups at visit 0, 1 and 6 ($p < 0.377$; $p < 0.213$; $p < 0.727$; $p < 0.389$; $p < 0.205$; $p < 0.634$). The group <5th percentile at visit 0 and 6, no statistical difference was seen ($p < 0.462$; $p < 0.616$; $p < 0.513$; $p < 0.814$). The trend in IgA levels was high levels at visit 0, an increase at visit 1 followed by a return towards normal levels. Significant difference was seen with the group A and B <5th percentile children. Group A, IgA increased substantially in comparison to group B ($p < 0.032$). For HFA, a similar picture is seen, group A IgA levels increase at visit 1, in-comparison to group B children who show a substantial decrease in IgA levels ($p < 0.018$). Figures 3.32 and 3.33 provide a visual representation of IgA levels for both groups by percentiles and WFA and HFA.

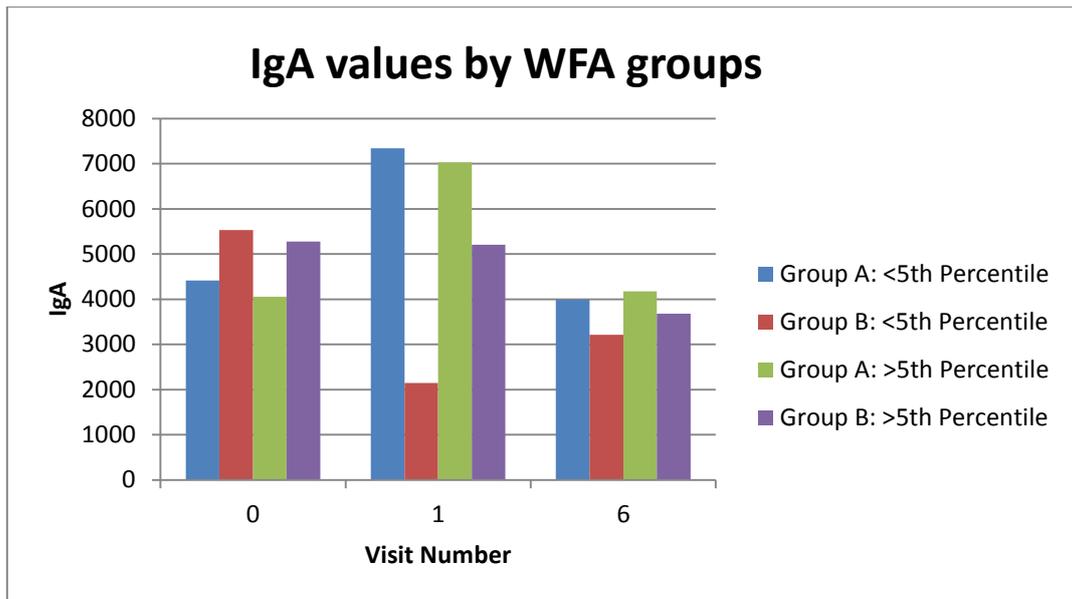


Figure 3.32: Comparison of IgA values by group and percentile for WFA

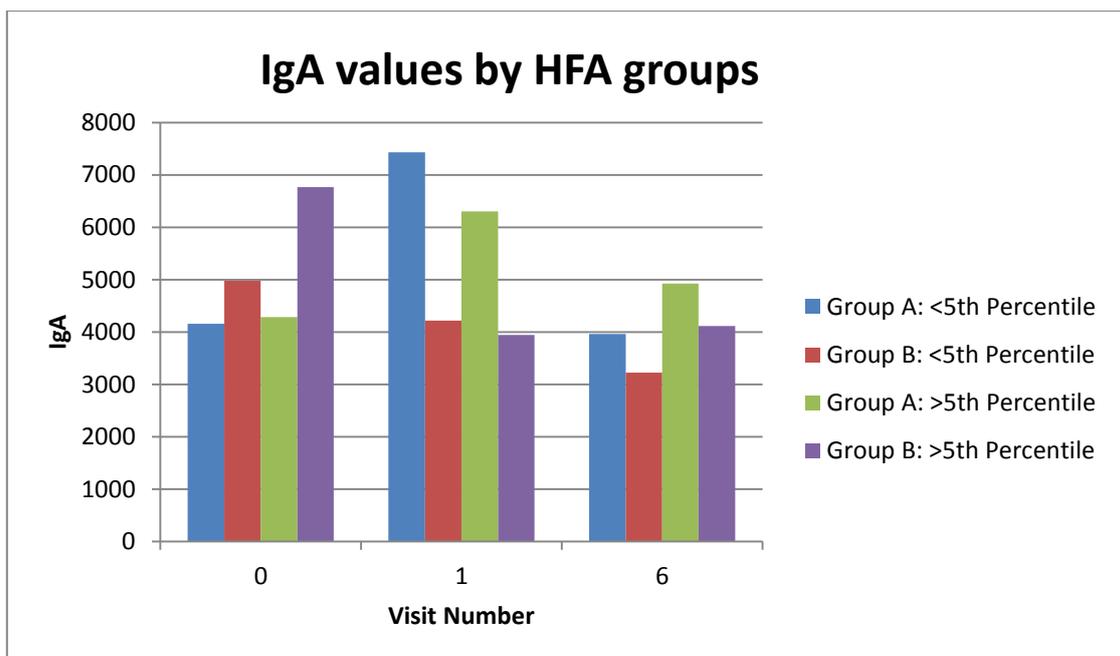


Figure 3.33: Comparison of IgA values by group and percentile for HFA

3.5 ADVERSE EVENTS

Table 3.38 lists the serious adverse events that occurred during the study (while the child was receiving the nutritional supplement). These occurrences resulted in hospitalisation of study participants. These events were reported to the Ethics Committee’s Board within 7-days of the occurrence. All serious adverse events were deemed to be unrelated to the nutritional supplement consumption.

Table 3.17: List of Serious Adverse events that required hospitalisation

Hospitalised	Adverse event
Yes	Child admitted to hospital with measles, LRTI, acute gastroenteritis and minimal respiratory distress
Yes	Mother reported coughing and fast breathing
Yes	Father reported history of rigours, progressive malaise and vomiting
Yes	Pneumonia and measles

During the study, the caregivers would report any untoward illness that occurred during the previous month (Table 3.37). These adverse events resulted in a doctor's visit or clinic visit. These visits were documented in the CRF of the individual participant. The blood or fecal related (excluding diarrhoea) events was identified through the routine blood and fecal tests carried out for the study purposes. These events were reported in the Ethics Committee's annual report. All adverse events were deemed to be unrelated to the nutritional supplement consumption.

Table 3.18: List of Adverse events that required a visit to the doctor or the clinic

No. of children	Adverse event
4	Anaemia
1	Burns
1	Cellulitis
1	Chickenpox
1	Dehydration
7	Eye discomfort (includes: Conjunctivitis, watery eyes)
21	Ear discomfort (includes: Otitis media, discharge from ear, abscess on back of ear)
59	Respiratory/Nasal discomfort (LRTI*,URTI*, cough, fever)
49	GIT discomfort (includes: Worms, Shigella, diarrhoea)
5	Macrocytosis
6	Mouth sores (includes: Herpes, cold sores)
6	PPE*
41	Skin discomfort (includes: Ringworm, rash, urticaria, dandruff)
1	Sores in nose
1	Severe Normocytic anemia
7	Tinea Capitis/Tinea Versicolor
6	Tonsillitis
2	Ulcer
4	Urinary Tract Infection

* LRTI: Lower respiratory tract infection; URTI: Upper respiratory tract infection; PPE: Pruritic papular eruption

CHAPTER 4: DISCUSSION

The aim of this study was to determine the effect of a complete nutritional supplement given in addition to daily food intake, on anthropometric measurements (weight and height) and immune markers on a cohort of HIV-positive children aged 24-72 months. Furthermore, the effect of addition of prebiotics, probiotics and DHA to this supplement on the weight, height and immune marker, C-reactive protein, was explored. As was mentioned in the introductory chapter, there is no evidence similar to the aims of this study. Interventions have been implemented, but the aims of those studies were rather focused on in-patient outcomes or the acceptability of a specific type of nutritional intervention^(43,68). Other studies also focused on children with only malnutrition or only children diagnosed with HIV^(68,69). This study therefore will be providing new knowledge on children infected with HIV, receiving ART and who are underweight and how best to address poor growth and strengthening the immune system.

4.1 KEY FINDINGS

For the 2-year age-group, the key findings showed that weight gain, as measured by weight-for-age z-scores, was statistically significant from visit 0 – visit 6. The absolute weight gain over time for the 2, 4 and 5 year age-groups was also statistically significant. Children presenting with a WAZ below -2SD gained more weight than the -1 and -3 SD. Statistically significant absolute height gain over time was achieved by the 2, 4 and 5 year age-groups. Height gain in children with a HAZ below <3SD, was statistically significant compared to height gain in children <-1SD and -2SD. For the cohort of children receiving the complete nutritional supplement containing prebiotics, probiotics and DHA, only <-3SD HAZ was statistically significant for group B compared to group A. Group A WAZ for the >5th percentile children had further improvement in CRP levels compared to group B.

All biomarkers (CRP, calprotectin and IgA) were reduced significantly by visit 6.

Nutritional supplementation was beneficial, through the reduction of severity of inflammation, which the outcomes of this study have shown.

4.2 IMPACT ON ANTHROPOMETRIC MEASURES

Children within the 2-year age-group showed significant improvement in weight-for-age z-scores at visit 6, however the 2, 4 and 5 year age-groups showed significant improvement in absolute weight.

Absolute weight or height measures provide centimetres or grams/kilograms of growth within a given population. These values are reported as means for that specific population or cohort, but with no comparison to a well-nourished reference population. In contrast, the z-score values for weight or height compares the absolute weight/height measures with the median values of a well-nourished reference population of the same age and gender (WHO 1995). The weight-for age z-score for the 2-year old age-group showed that, when compared to a well-nourished reference population, a significant improvement in weight gain was seen when these children were supplemented with an enriched nutritional supplement. This is a significant finding when it is considered that these children were HIV-positive and were underweight at visit 0. The statistical significance of the absolute weight gain for the age-groups 2, 4 and 5 are clinically important. This study showed that providing a supplement to underweight HIV-positive children, in addition to their habitual daily intake, can improve weight on a monthly basis.

When grouping the children into those <5th percentile WFA and those between the 5th – 15th percentile WFA, the growth during the 6-months supplementation was statistically significant in the children <5th percentile WFA. These findings are important bearing in mind that the rate of growth is determined by nutrition, chronic disease and endocrine function^(70,71). Growth retardation occurs due to poor nutrition and states of inflammation. If nutritional rehabilitation occurs, catch-up weight can be reached. This catch-up can be rapid or ongoing. However, this growth will not be according to the normal weight-for-age specific to the child, rather a lower percentile for weight will be followed. This percentile will not be in-line with their specific age-group but rather a new growth curve⁽⁷⁰⁾. Other studies refer to it as a re-adjustment of growth process if the situation is favourable i.e.: good nutrition and a supportive environment^(72,73). Ashworth et al reported in their review that it was not unusual to see increased rates of weight gain if the child was severely malnourished. The rate of weight gain can be as much as 20 times faster than for the healthy normal child⁽⁷³⁾. The clinical significance of this finding is that children with more pronounced malnourishment will be much more sensitive or receptive to nutritional support and thus more likely to show catch-up growth.

Cape Town has a population of mixed race (Coloured) 42% and a black African population of 39%. Seventy eight percent (78%) of the population living in Cape Town reside in formal dwellings, also referred to as urban informal settlements⁽¹¹⁾. These socio-demographic characteristics are important when considering the South African context. The SANHANES 2015 report found that the African black population race group had the highest rate (30.3%) of food insecurity followed by the coloured population (13.1%). Furthermore, the largest

percentage of participants who were food insecure were residing in the urban informal areas (32.4%) and the rural formal areas (37.0%)⁽¹⁵⁾. The majority of the children from the study reported in this thesis originated from food insecure communities and urban informal areas. Children were either black African or from the Coloured population. When we consider the nutritional intake at time point 0, these children were consuming sufficient nutrients for a well-nourished, HIV-negative child. However, these children were not only underweight (which according to the SA guidelines for MAM requires additional energy via the NTP of 420kj/kg per day⁽³⁹⁾), but HIV-positive and receiving ART. ART increases the resting expenditure of an individual, which results in increased energy requirements of up to 50% - 100%⁽¹⁴⁾. With the introduction of the complete nutritional supplement, the nutritional intake was substantially increased over a 6-months period. The children were at a growth deficit at visit 0 and they experienced catch-up weight during the period of this study. However, it is important to note that if the cause of the growth deficit persisted for a long duration, then the weight gain may increase but still remain below the normal growth curve and not reach catch-up. In-fact these children will then establish a new growth curve according to their individual growth, referred to as an energy equilibrium phase⁽⁷⁰⁾, which is made up of the child's adjusted genetic growth potential and the present energy intake. Trehan et al found in the management of community-based care for severely malnourished children who are HIV positive, that children who are HIV-positive and malnourished invariably have a reduced ability for nutritional recovery in comparison to their HIV-negative counterparts. They also reported that, those who do recover take longer to achieve full nutritional recovery^(22,70). It is important to consider that these children were provided with the complete nutritional supplement for 6-months, which led to statistical significant improvements of weight over the 6-month period. If they were provided with the complete supplement for a longer duration and/or followed for a longer period, the weight gain could potentially have resulted in catch-up growth/nutritional recovery on the mid- to long-term.

This study considered both the percentiles and z-scores to analyse the growth of the children. Growth charts, which identify a specific percentile of growth for a child are not designated as the sole diagnostic measurement, rather it should be used to assist in forming an overall clinical impression of the child being measured. Z-scores provide a comparison group to address the hypothesis of the test, i.e.: whether supplementation improves growth for underweight HIV positive children. It is important to remember that the z-score analysis is a comparison between this cohort (underweight; HIV positive) and a cohort of healthy children, with no HIV disease⁽⁵³⁾.

Weight gain across age-groups produced interesting findings, where the 2 and 5 year old age-groups experienced the highest incremental gain from visit 0 to visit 6, whereas the 3 and 4 year age-group experienced marginal increase in weight from visit 0 to visit 6. These results are in-line with the somatic growth from birth to adulthood. In the first 2-years of life the rate of weight gain is rapid and this effect levels off between 2-3 years. Physiological growth continues according to the percentiles from 2-8 years, with 3-5 years experiencing a slow but stable growth improvement compared to the 2-year olds which experience haphazard weight gain dependent on clinical and nutritional factors. The adolescent growth spurt then starts at 8-years of age⁽⁷⁰⁾. This is an interesting finding which is not clearly understood. It could be that providing a supplement as an intervention to younger children produces a better response, or it could be that the supplement aided weight gain which resulted in normalising of the newly established growth trajectory.

Ashworth et al reported in their review that only if the child presented as being wasted (weight deficit is greater than the length), then the weight gain will be faster than the height gain until the appropriate weight-for-height was reached. After this, weight and height will continue to increase at the same rate⁽⁷³⁾.

The children in the category for HAZ <-2SD and <-3SD showed clinically significant improvements in height. The children in the cohort, irrespective of supplement type, gained in height, where they moved from the category of severely stunted (<-3SD) to the category of moderately stunted (<-2SD). They also moved from the category of moderately stunted (<2SD) to the category of mildly stunted (<-1SD). These are fundamental findings as stunting is more difficult to reverse in age-groups >2 years and the longer the duration of the stunting^(1,4). Absolute height measures were found to be statistically significant, as improvement in height was shown from visit 0 to visit 6. At visit 0 both boys and girls were moderately stunted, and at visit 6, only the boys and the girls age-group 3 years remained moderately stunted. However, the girls in the age-group 2, 4 and 5 moved from the moderately stunted to mildly stunted category. The same argument can be considered for height as for weight. All the children were stunted to different degrees at the onset of the study. The short duration of the additional intake, the HIV-positive status and the increased nutritional needs, did not allow for a full recovery of height gain. However, a longer duration of supplementation potentially could achieve recovery of height, because this study showed different degrees of improvement of height gain for all age-groups. Again it can be seen in the data that the age-group 2 and 5 years had the most significant improvement in height gain. An interesting finding was that when comparing gender, the girls across age-groups all experienced a greater improvement in height gain compared to the boys. In other words, the

boys remained more stunted in comparison to the girls and the boys did not grow as rapidly compared to the girls. The SANHANES report showed a trend in growth amongst SA children that could possibly explain why the girls grew better than the boys in this cohort of children. The report found that boys were more stunted, wasted and underweight than girls⁽¹⁵⁾. The SANHANES report found that regardless of HIV-status, in the age-group 0-3 and 4-6 years the prevalence of stunting, wasting and underweight was 26.5%, 11.9% and 9.4% respectively⁽¹⁵⁾. The causes of stunting and low height-for-age z-scores can be attributed to the HIV disease and undernutrition. The difference across genders is related to the physiology of somatic growth. The skeletal maturation of girls is 4-6 weeks more advanced than boys, this however stabilises from 4-5 years onwards⁽⁷⁰⁾. Evidence has linked stunting to the low-grade inflammation associated to HIV disease, low levels of education of mothers of stunted infants, and feeding practices among HIV-positive mothers^(1,12). Nutritional interventions alone cannot address stunting for individuals or communities. There are underlying factors that contribute to stunting and therefore all factors need to be addressed in conjunction with nutritional supplementation for true recovery of weight and height parameters to be attained.

4.3 Impact on immune markers

A further aim of this study was to investigate the effect of nutritional supplementation on immune markers. HIV directly causes impairment of the immune system, this leads to malnutrition. Malnutrition in turn affects immune function and worsens the HIV. This results in the HIV infection progressing faster to AIDS. HIV causes immune impairment through a variety of mechanisms, these include reducing the number of CD4 cells, reducing cytokine production which directly impacts inflammation and the ability of the immune system to fight off infections. HIV infection leads to malnutrition through the direct impacts on the GIT. This is done through the ability of the HIV infection to impair the intestinal villi which reduces absorption capacity and increases permeability. By improving the nutritional status of the child, the child is more able to fight off opportunistic infections, because the immune system can be rehabilitated/strengthened^(20,74).

This study found a statistical significance in the visit 0 CRP levels compared to the visit 6 CRP levels. At visit 0 the CRP levels for the cohort of children was above the normal range and after 6 months of supplementation the CRP levels decreased and was within the normal range. This finding should be interpreted cautiously as there was a time lapse between the visits and the small sample size of children that had a CRP test at visit 0 and at visit 6. This relative improvement in CRP levels may be associated with an improved anthropometric status of the children.

High levels of CRP are caused by infection and chronic disease⁽²⁵⁾. Individuals with elevated CRP were found to have an infection and were underweight⁽²⁰⁾.

When comparing children <5th percentile and children >5th – 15th percentile for WFA and HFA, similar outcomes were noted. Both groups had elevated CRP at initiation and after 6-months of supplementation, CRP levels normalised. For both WFA and HFA >5th – 15th percentile and <5th percentile, no group was statistically more significant in change in CRP levels overtime than the other. The change in CRP levels for both was similar.

We can therefore conclude from the evidence that the children in this study had a chronic infection (HIV) and were underweight and therefore experiencing systemic inflammation. Inflammation negatively impacts nutritional status by separation of minerals, poor absorption due to recurrent diarrhoeal infections which are a result of bacterial infections⁽²⁰⁾, nutrient losses and inhibits the use of nutrients by the body⁽⁷⁵⁾. In this study, nutritional supplementation could potentially have improved nutritional status through the provision of additional nutrients which possibly improved the quantity of energy intake thus bringing about reduction in inflammation.

Calprotectin is a reliable and effective marker to identify gastrointestinal inflammatory activity⁽⁷⁶⁾. The findings of this study show a statistically significant reduction in faecal calprotectin levels after nutritional supplementation. At visit 0 the cohort's median for calprotectin was far higher than the reference range aligned with CRP levels. This is similar to findings from other studies including a study in Uganda which found that HIV-positive children had a median faecal calprotectin higher than the reference ranges. Another study showed HIV-positive children in their cohort having higher levels of biomarkers of vascular dysfunction in comparison to a cohort of healthy urban children. They reported that biomarkers specifically related to inflammation and endothelial dysfunction was higher in the HIV-infected cohort compared to the control cohort^(28,30).

Both CRP and calprotectin have been linked to poor growth in children. In Zimbabwe, CRP was found to be significantly higher in stunted children⁽¹⁾ and in another study children presenting with poor growth were found to have higher faecal calprotectin levels compared to children who had normal growth⁽⁷⁶⁾.

When comparing children in the <5th percentile and >5th – 15th percentile by WFA and HFA both groups experienced a marked elevation of calprotectin levels at month 1, then a reduced calprotectin level by month 6. For both WFA and HFA >5th the percentile and <5th – 15th percentile, no group was statistically more significant in change in calprotectin levels

overtime than the other. The change in calprotectin levels for both was similar. This finding can be explained by immunodeficiency caused by the HIV infection inhibiting the usual T-cell response to a pathogen at the time of it entering the system. Once immune recovery occurs, an inflammatory response that could lead to immune reconstitution inflammatory syndrome (IRIS) may occur. This has been found in studies of ART initiation, refeeding syndrome and severe malnutrition rehabilitation^(75,77). A possible explanation for the marked elevation at month 1 could be that the supplement corrected or improved the immune status and resulted in immune activation. Homeostasis was recovered over the 6-months intervention period and the calprotectin levels stabilised as the immune response was down regulated.

IgA is known to be increased during an infection, reflecting immune defence function of the host⁽³²⁾. The children in this cohort at visit 0 had levels of IgA far above the normal range. At visit 6 the IgA level had reduced, although not within the normal range. However, the reduction in IgA level was statistically significant. This data may suggest that prior to introduction of the complete supplement, the gut epithelium lining and integrity was altered, this could have induced an increased translocation of gut bacteria and provoked host immune response as illustrated by elevated IgA and inflammatory status. In such HIV-positive and malnutrition conditions, this vicious circle is maintained and contribute to the vulnerability of the subject to various infections and diseases^(32,33). After 6-months of nutritional supplementation, which resulted in improvement of the nutritional status of the children, both GIT and systemic environments improved as the inflammation was reduced. When comparing the >5th – 15th percentile group of children for WFA and the <5th percentile group of children for HFA, both experienced an elevated IgA, greater than the elevated level at visit 0. This finding can be explained as for the calprotectin. As the immune system recovered, it was able to function effectively to clearing out pathogens which included an inflammatory response, but as the mucosal environment improved so the inflammation reduced, thus decreasing the elevated IgA levels. For both WFA and HFA >5th – 15th the percentile and <5th percentile, no group was statistically more significant in change in IgA levels overtime than the other. The change in IgA levels for both was similar.

The results of this study were predominantly influenced by the underlying HIV-disease coupled with the malnutrition. Significant improvements in CRP, IgA and calprotectin were an outcome of the study which potentially indicates a reduction in the HIV and malnutrition associated impacts in this cohort of children.

4.4 Impact of supplementation with additional nutrients

Objective three was aimed at exploring whether supplement B, which included the DHA and probiotics, had a greater impact on growth and immune markers compared to supplement A.

The $<-3SD$ HAZ group B was the only parameter where group B improved height more significantly than group A. For WAZ, WFA and HFA, both showed improvement in weight and height, but not different to the other except in the HAZ $<-3SD$.

Overall both supplements impacted growth in children positively. Supplement B tended to have a more marked impact on growth compared to supplement A. Supplement B also had a better effect if the child was moderate to severely stunted.

The children in group A WFA $>5^{th}$ – 15^{th} percentile, had a statistically significant improvement in CRP compared to group B. And for the $<5^{th}$ percentile children in the group B, a statistically significant reduction in IgA was seen in comparison to group A. All other biomarkers were improved, but the improvement was not statistically different between the two groups.

Children in group A, seemed to experience rapid increase then decrease in calprotectin and IgA. Where group A showed a reduction in biomarker levels, this change was rapid, in comparison to the group B which had a gradual decrease in biomarker levels. Interpretations on these findings are made with caution, as reasons are unclear. These findings could be related to the children's immune system, it could be that the group A children were severely immunosuppressed and supplementation triggered an immune response. In comparison, group B children did not experience IRIS, and so it seems that their reduction in biomarker levels was gradual.

Overall both supplements were associated with improvement in immune markers. Children consuming Supplement A had a rapid improvement where children consuming supplement B had a more gradual improvement. In the case of HIV and underweight children, a slower and more controlled change would probably be better clinically. Sudden changes could result in severe immune reconstitution, hyper-inflammatory state or refeeding syndrome, which could potentially lead to death. Similar to growth parameters, supplement B had a greater impact on the children $<-3SD$ for HAZ. This may allude to superiority of supplement B possibly; however, further research is needed to confirm this.

Introduction of the nutritional supplement provided a substantial increase in total energy, other macronutrients and micronutrients. Improvement in growth was noted, but catch-up weight was not achieved. This could be attributed to the HIV disease and the increased

requirements due to the ART⁽¹⁴⁾. Other studies have found that malnourished HIV-positive children have a reduced ability to achieve nutritional recovery compared to their HIV negative counterparts⁽²²⁾. A readjustment of growth will occur, positioning the child on a close to normal growth trajectory, if the conditions for growth are supported (good nutrition and better environments)⁽⁷²⁾.

The findings of this study indicate the positive effects of nutritional supplementation as an intervention for supporting growth and immune markers in HIV-positive underweight children. These findings are similar to two intervention studies in Africa, namely in Malawi and in Zambia. In Malawi, supplementing with an enriched supplement was significantly more effective in improving nutritional status of HIV-positive and HIV-negative children when compared to traditional food such as blended maize flour. In Zambia, HIV-infected children receiving an amino-acid based elemental feed experienced increased weight in comparison to the children receiving the standard nutritional rehabilitation⁽¹⁸⁾.

In relation to the use of nutritional supplementation as an approach to addressing HIV disease progression, malnutrition and food insecurity for children, this study shows that nutritional supplementation can positively support growth and immune markers. The mechanism through which nutritional supplementation exerts its effect, is thought to be the provision of additional energy and micronutrients, which collectively improves nutritional status. An improved nutritional status will then contribute to improved growth and immune markers⁽⁷⁸⁾. A review by Aberman et al considered evidence on nutritional supplementation (NS) to individuals and community, safety-nets targeted at households (food parcels, cash or vouchers) and cash transfers to improve food security in the response to the AIDS epidemic. Each intervention reported benefits. For example, NS to individuals and communities reduced weight loss, prevented postnatal transmission of HIV, improved BMI and lean body mass. Supplementing with high dose micronutrient showed benefits such as improved CD4 cell count and survival among people living with HIV. Supplementation studies in SA have also shown benefits in nutritional status and quality of life, although these impacts were small. Food parcels, vouchers or cash interventions only improved drug adherence, and not weight gain and CD4 cell response. A study in Kenya reported that when households were provided with food parcels and nutrition education, recipients reported increased dietary diversity and diet quantity and improved health status of household members especially young children⁽⁷⁹⁾. These studies were either focused on adult populations or households, with limited data on children as the target group. In addition, these studies were done on individuals and families affected by HIV/AIDS and the HIV positive individuals were on ART. ART improves immunological and nutritional outcomes of HIV infection in children. A few

documented studies have shown nutritional supplementation along with ART have resulted in better outcomes among children. For adults on ART, nutritional supplementation did not significantly increase weight gain⁽⁷⁹⁾.

The recommendation would then be that a combination of strategies is needed, the better one of which may be nutritional supplementation in-conjunction with ART. However, other factors such as nutrition education to caregivers and young women pre-conceptually as well as support from agricultural interventions would need to be included to provide a more holistic approach to managing the HIV infected child. These approaches would also address the prevention of malnutrition, household food insecurity and improved nutritional status of children⁽⁷⁹⁾.

4.5 LIMITATIONS

The secondary analysis on a small blood sample size of this study was a limiting factor for the testing of immune markers. Challenges associated with collecting blood and faecal samples from young children accentuated this limitation. This resulted in the study not being powered enough to support efficacy.

The small sample size per randomized group in the secondary analysis also made it difficult to identify whether the enriched supplement with probiotics, prebiotics and DHA had a significantly different effect on growth and immune markers.

The uncertainty or confirmation of whether the child came from a food insecure home may have skewed the reporting for the nutritional intake and the consumption of the enriched supplement. The investigators received verbal confirmation from caregivers that the children who were enrolled on the randomized trial were the only individuals who consumed the supplement. Although investigators counted the empty returned tins of the supplement and logs were kept, they relied heavily on the verbal feedback from the caregivers regarding compliance.

Although assessing the nutritional intake of the children was not a set objective for this thesis, it is important to consider the limitations associated with the dietary intake data. The dietary intake data was collected using the QFFQ which has its limitations which include; over or underestimates due to limitations in assessing quantities well as recall bias, despite the measures that has been taken to address these limitations. These could have been reasons for the increased dietary intake of children originating from apparently food insecure communities⁽⁵¹⁾.

4.6 CONCLUSION AND RECOMMENDATIONS

Although the findings of this study provide new evidence, it should be interpreted with caution. The apparent supplement induced support on growth and improvement in immune markers can be attributed to a combination of factors. These factors include normal development, adaptation to ART, reduction of food insecurity and the social aspects emanating from the clinical trial.

The table 4.1 below documents the required energy needs for children who are either malnourished, HIV positive or HIV positive and receiving ART^(14,42).

Table 4.1: Energy Requirements for children with specific conditions

Nutritional requirements	Co-morbid conditions
30% additional energy required	Malnutrition
10%-50% additional energy required	HIV (asymptomatic/symptomatic)
50% - 100% additional energy required	HIV on ART

The nutritional recommendations for HIV positive children, underweight children and children on ART in total would imply an intake beyond what the child could consume daily. Although the nutritional supplement in this study provided additional energy, it still did not necessarily meet the child's required intake. It is difficult to fully meet the nutritional requirements of these children, so this intervention merely afforded an opportunity to attempt to increase nutritional intake and to establish a degree of tolerance and compliance, which has shown some success. In developing countries, particularly in Africa, HIV-infected children are affected by a multitude of factors and a single nutritional supplement cannot alone ensure nutritional recovery. Factors such as accessibility to ART, food insecurity, child care and stimulation, access to appropriate medical care and maternal factors also need to be addressed simultaneously for the child to receive the full benefits of nutrition supplementation for possible full recovery.

It should also be borne in mind that the HIV disease itself continues to negatively impact the nutritional status of an HIV positive child, therefore long-term nutritional supplementation will be required to assist and support the maintenance of adequate growth and support the immune system.

This study identified three important findings in terms of clinical impact. Firstly, a well-balanced and complete nutritional supplementation should be provided as early as possible for children. This study showed that the younger the child, the greater the effect. Secondly, the more malnourished and stunted the child is, the greater their response in weight gain and height gain on a nutritional supplement. And lastly, providing nutritional supplementation

for 6-months supported growth and decreased immune inflammatory markers. Whether these effects can be sustained over a longer duration of supplementation needs to be further investigated.

Recommendations for future studies:

- Recruitment of a larger sample or ensuring sufficient biological samples to investigate a reliable and statistically significant effect for all primary and secondary analysis is needed.
- Supplementation of HIV positive children should be conducted over a longer period to identify the sustainability of catch-up growth and nutritional recovery.
- Supplementation should be provided in children early (0 – 3 years and 4-6 years) as stunting begins *in utero* and weight deficits can occur as early as 2-3 months of age. By providing an enriched supplement earlier in the lifecycle can potentially address stunting and underweight before it becomes irreversible.

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ADDENDA

Addendum 1: Study information leaflet for caregivers of participants

Purpose of the study:

- To see if a nutritional supplement will help your underweight child gain weight (Age of child should be 24 – 72 months old)



1

The study involves:

Giving your child the nutritional supplement or drink 2 times a day for 6 months.



For example in the morning and evening.



2

Weigh and measure your child:

Your child's weight and height (length) will be taken once a month for 6 months.



Weighing a child



Measuring the length of the child

3

Diet History

At some of your study visits we will ask you questions about

- The types and amounts of foods your child eats,
- How you cook the food,
- How often your child eats these foods.



4

Blood tests:

- A total of 7 mls (1½ teaspoons) of blood will be taken once a month for 5 months.
- Different blood tests will **be** done on the blood that is collected.



5

Urine tests:

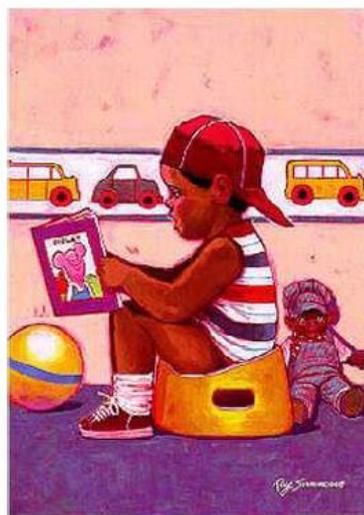
- Urine will be collected once a month for 3 selected months.
- Urine will be taken over a period of 5 hours after the child drinks a special fruit juice.



6

Stool tests:

- A study nurse will collect a stool sample from the child every month for 5 selected months.
- If the child has diarrhoea, the study nurse will collect extra stool samples from the child.



Child using a potty

7

Benefits of taking part in study:

Your child **may** :

- Gain weight,
- Have less infections,
- Have improved immune system to fight diseases and infections
- Have an improved digestive system that uses food more efficiently,
- Have no benefit except get a nutritious supplement to drink.



8

This is a voluntary study:

- To participate in this study is voluntary.
- If you refuse to participate, it will not affect your child's medical care at the clinic in any way.
- Only the investigators will have access to the information gathered.

9

If child gets sick while taking part in study:

- Medical treatment will be offered for free by a doctor at the Tygerberg clinic.
- Please note that the nutritional supplement that the child will drink is safe, has been extensively studied.
- There is no or minimal risk in taking part in the study.

10

Minimal risk involved maybe:

- Needle pricks when blood is been drawn once a month for 5 months by a qualified professional nurse. Your child may experience some pain and bruising with the needle prick.
- HIV positive children are more prone to infections than other children. Should your child develop any infection during the study, the child will be treated with appropriate therapy.

11

Transportation to study site

- For each time you bring your child to the study site, all transport costs will be covered.
- If you stay in the hospital for the study over lunch, a finger snack will be provided for you and some money will be given.
- You will not be paid any money to take part in the study.

12

The study is conducted at:

- Tygerberg Hospital, 8th floor, East side Room H64, near the J8 ward also called Kid Cru.
- Hospitals taking part in this study are Tygerberg hospital, Paarl hospital and Groote Schuur hospital.

13

If you have any questions, please
contact:

- Professor D Labadarios at 082 772 6961

14

Addendum 2: Consent Form

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM FOR USE BY PARENTS/LEGAL GUARDIANS AT TYGERBERG HOSPITAL

TITLE OF THE RESEARCH PROJECT:

Catch Up Weight in Underweight HIV positive children supplemented with an oral supplement

REFERENCE NUMBER: Trial Number 05-16-CLI

PRINCIPAL INVESTIGATOR: Prof D Labadarios

ADDRESS: Department of Human Nutrition

P.O Box 19063

Tygerberg

7505

CONTACT NUMBER: 082 772 6961

Your child _____ is being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how your child could be involved. Also, your child's participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you or your child negatively in any way whatsoever. You are also free to withdraw him/her from the study at any point, even if you do initially agree to let him/her take part.

This study has been approved by the **Committee for Human Research at Stellenbosch University, University of Cape Town Research Ethics Committee, Tygerberg Academic Hospital and Paarl Hospital Ethics Committee. The study is also registered at the Department of Health.** This study will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research. Children who may participate in the study will be from Tygerberg, Groote Schuur and Paarl Hospitals.

What is this research study all about?

- Purpose of the study is to explore if a nutritional supplement will help your underweight child gain weight.
- The study will involve
 - Giving your child a nutritional supplement/drink two times daily.
 - Every month (for 7 months) your child will undergo the following tests or measurements:
 - Weight, height measurements.
 - Blood tests. A total of 7 ml (1.5 teaspoons) will be drawn from your child. The blood will be tested for different blood components.
 - Urine test. Urine will be taken over 5 hours after the child drank a special drink. The urine will be tested

to see how well your child's body uses the food that is eaten.

- Stool sample. You will be asked to collect a stool sample. A special container will be given for the purpose of collecting the stool at home prior to visiting the hospital. If the child is in hospital, the nurse will collect the stool sample using the special container.
- For scientific reasons, all children in the study will be assigned to receive one of two similar nutritional supplements. This will be done in a way that neither the study participant nor the investigator will know who receives which supplement. All children will have an equal chance of being assigned to any of the two product groups. All children in the study will undergo all the tests described above.

Why has your child been invited to participate?

- Your child has been invited to participate in the study because he/she is underweight for his/her age. This nutritional supplement/drink may help him/her gain weight. .

What will your responsibilities be?

Your responsibilities as a parent in this study are the following:

- Ensure that your child drinks the nutritional supplement two times a day (a total of two cups).
- Ensure that your child consumes his/her normal meals daily.

- Bring the child to the clinic every month as requested by the study and clinic staff.
- Collect a stool sample and bring it to the clinic, when necessary.

Will your child benefit from taking part in this research?

Your child may benefit from taking part in this study by:

- Gaining weight.

Other potential benefits that will be tested in this study are:

- Reduced frequency of infections.
- Improved immune system that helps your child fight diseases and infections.
- Improved digestive system that uses food that is eaten more efficiently.
- May not gain weight but have a nutritious supplement to drink.

Are there any risks involved in your child taking part in this research?

- The risks involved in this study are minimal. They are limited to needle pricks when blood is drawn once a month (for 5 months) by a qualified professional nurse. Your child may experience some pain and bruising with the needle prick.
- HIV positive children are more prone to infections than other children. Should your child develop any infection during the study, the child will be treated with appropriate therapy.
- **If you do not agree to allow your child to take part, what alternatives does your child have?**
 - This study is purely on a voluntary basis. Refusal to participate in the study will not affect your child's medical care at the clinic where this study is being held.

- **Who will have access to your child's medical records?**

- All information received will be confidential and will be protected.
- The information collected will be published in a University Thesis and a scientific paper. However, there will be no mention of any names or any information that may identify your child.

The following people will have access to your child's medical records:

- Principle investigator (supervisor).
- Medical advisor to the study.
- Study assistants - who will enter the entire child's information in the computer for analysis. All names or information that may identify your child will be left out by the study assistants.

- **What will happen in the unlikely event of your child getting injured in any way, as a direct result of taking part in this research study?**

- If your child gets injured as a result of drinking this nutritional supplement, then the following will occur:
 - All adverse events will be documented.
 - Medical treatment will be offered by a doctor in the clinic at no cost to you.
- Please be assured that the nutritional supplement is safe and ingredients have been extensively studied.

Will you or your child be paid to take part in this study and are there any costs involved?

You or your child will not be paid to take part in the study, but transport costs will be covered for each study visit. A meal cost of R20 will be provided when you have to stay for the full day. Therefore, there will be no costs involved for you if your child does take part in the study.

Is there any thing else that you should know or do?

- You should inform your family practitioner or usual doctor that your child is taking part in a research study.
- You should also inform your medical insurance company that your child is participating in a research study.
- You can contact Prof D Labadarios at telephone 082 772 6961 if you have any further queries or encounter any problems.
- You can contact the Committee for Human Research at 021 938 9207 if you have any concerns or complaints that have not been adequately addressed by your child's study doctor.
- You will receive a copy of this information and consent form for your own records.
- If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the South African Medicines Control Council (MCC) at:

The Registrar; SA Medicines Control Council; Department of Health; Private Bag X828; PRETORIA; 0001; Fax: (012) 312 3105; e-mail: labusa@health.gov.za.

A. Declaration by parent/legal guardian:

By signing below, I (*name of parent/legal guardian*)
..... agree to allow my child (*name of child*)
..... who is years old, to take part in a research
study entitled: Catch up weight in underweight HIV positive children supplemented
with the supplement.

I declare that:

- I have read or had read to me this information and consent form and that it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to let my child take part.
- I may choose to withdraw my child from the study at any time and my child will not be penalised or prejudiced in any way.
- My child may be asked to leave the study before it has finished if the study doctor or researcher feels it is in my child's best interests, or if my child does not follow the study plan as agreed to.

Declaration that blood can be drawn:

Signed at (*place*) on (*date*)

200 .

.....

Signature of parent/legal guardian

.....

Signature of witness

Declaration to participate in the study:

Signed at (*place*) on (*date*)
200 .

.....
Signature of parent/legal guardian Signature of witness

B. Declaration by the investigator:

I (*name*) declare that:

- I explained the information in this document to
.....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understand all aspects of the research, as discussed above
- I did/did not use a translator (*if a translator is used, then the translator must sign the declaration below*).

Declaration that blood can be drawn:

Signed at (*place*) on (*date*)

200 .

.....

Signature of investigator

.....

Signature of witness

Declaration to participate in the study:

Signed at (*place*) on (*date*)

200 .

.....

Signature of investigator

.....

Signature of witness

C. Declaration by the translator:

I (*name*) declare that:

- I assisted the investigator (*name*) to explain the information in this document to (*name of parent/legal guardian*) using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the parent/legal guardian fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Declaration that blood can be drawn:

Signed at (*place*) on (*date*)
200 .

.....
Signature of translator

.....
Signature of witness

Declaration to participate in the study:

Signed at (*place*) on (*date*)
200 .

.....

.....

Signature of translator

Signature of witness

Addendum 3: Standard operating procedures for measuring weight in children

Weight

1. During the study the weight of the child must be taken every month.
2. Use an electronic platform scale that is accurate to 100g (0.1kg)
3. Calibrate the electronic platform scale using the 10kg empirical weight daily as well as after moving the scale.
4. If possible, the child's bladder should be emptied before taking this measurement.
5. Remove all outer clothing (overcoats, sweaters, shoes, heavy shirts), thus weigh the child in only his/her clean diaper or underwear.
6. Encourage the child to stand still in the middle of the scale without touching anything and with the body's weight equally distributed between both feet.
7. Record weight to the nearest 100g (0.1kg).
8. Two measurements taken in immediate succession should agree to within 100g (0.1 kg).
9. If it is not possible to weight the child on the platform scale, the child can be weighed while being held by an adult with the weight derived by the difference. Because this weight will be less accurate than desired, the method should be noted in the child's file.
10. Take three weights and calculate an average. Record the average (it will be used for analysing the weight change over the duration of the study).

Addendum 4: Standard operating procedures for measuring height/length in children

Length (Children 24 – 36 months)

1. At every study visit, i.e. every month, measure the child's length using a height board i.e. stationary headboard and moveable footboard that are perpendicular to the backboard.
2. Two people are necessary to measure the length.
3. Place the child in a supine position (lying on his/her back) on the headboard.
4. One person holding the child's head against the backboard, with the crown securely against the headboard and with the Frankfort plane perpendicular to the backboard.
5. This person also keeps the long axis of the child's body aligned with the center line of the backboard, the child's shoulders and buttocks firmly touching the backboard, and the shoulders and hips at right angles to the long axis of the body.
6. The other person keeps the child's legs straight and against the backboard.
7. This person then slides the footboard against the bottom of the feet (without shoes or socks) with the toes pointing upward, and reads the measurement.
8. The footboard should be pressed firmly enough to compress the soft tissues of the soles but without diminishing the vertebral column length.
9. Length should be recorded to the nearest 0.1 cm using a consistent unit over repeated measurements.
10. Use gentle restraint to keep a crying child properly positioned during measuring.

11. When this is not possible, the best estimate should be recorded with a notation of the circumstance.
12. Take the measurement three times. Record the average (it will be used for analysing the height change over the duration of the study).

Stature (Children 37 – 72 months)

1. At every study visit, i.e. every month, measure the child's stature using a stadiometer.
2. Place the stadiometer on a hard, vertical surface.
3. The child should be barefoot i.e. no shoes, only socks.
4. The child should have minimum clothing (overcoats, sweaters, shoes, heavy shirts, head dressing are removed) to enable correct positioning of the body to the stadiometer.
5. Hair ornamentation may have to be removed if this interferes with the measurement.
6. The child should stand with heels together, arms to the side, legs straight, shoulders relaxed, and head in the Frankfort horizontal plane.
7. Heels, buttocks, scapulae (shoulder blades), and back of the head should, if possible, be against the vertical surface of the stadiometer. (At least 2 to 3 points should touch, if all is not possible).
8. Just before the measurement is taken, the subject should inhale deeply, hold the breath, and maintain an erect position while the headboard is lowered on the highest point of the head with enough pressure to compress the hair.

9. The measurement should be read to the nearest 0.1 cm using a consistent unit over repeated measurements and with the eye level with the headboard to avoid errors caused by parallax.
10. Take the measurement three times. Record the average (it will be used for analysing the height change over the duration of the study).

Addendum 5: Quantitative food frequency questionnaire

CATCH UP WEIGHT IN UNDERWEIGHT HIV* CHILDREN SUPPLEMENTED WITH AN ORAL SUPPLEMENT

Subject Number: Birth Date: Interview Date:

Child's name: _____ Interviewer: _____

QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

Greeting

Thank you for giving up your time to participate in this survey. We would like to find out what your child, aged 24 – 72 months, usually eat and drink. This information is important to know as it will tell us if children are eating enough, of the right foods, and if they are healthy.

Please think carefully about the food and drinks the child, that has been identified as a participant in this study survey, have consumed during the past 6 months. I will now go through a list of foods and drinks with you and I would like you to tell me:

- If the child eats these particular foods,
- how the food is prepared (by you or the child's caretaker),
- how much of the food the child eat at a time, and
- how many times a day the child eat it and if he or she does not eat it every day, how many times a week or a month it is eaten?

To help you to describe the amount of a food, I will show you models of different amounts of the food. Please say which model is the closest to the amount eaten, or if it is smaller, between sizes or bigger than the models. Amounts must be reported as cups (c), tablespoons (T), serving spoons (SP) or teaspoons (t).

- THERE ARE NO RIGHT OR WRONG ANSWERS.
- EVERYTHING YOU TELL ME IS CONFIDENTIAL.
- IS THERE ANYTHING YOU WANT TO ASK NOW?
- ARE YOU WILLING TO GO ON WITH THE QUESTIONS?

QUESTION	YES	NO	REMARKS / OTHER			
1. Are you the mother of the child?	1	2	If no, please specify your relationship to the child:			
2. Does the child follow any special diet?	1	2	Don't Know			
			Diabetic	Silmming	Allergies	Other
			1	2	3	4
3. Has the child eaten away from home during the last week? Specify the number of times and place.	1	2	Don't Know			
			Number: Place/s*:			

*May be more than one place;

4. Does your child eat breakfast	Regularly (4 or more times a week)	Sometimes (1-3 times a week)	Never
	1	2	3

INSTRUCTIONS TO FIELDWORKERS:

CIRCLE THE CHOSEN ANSWER AND FILL IN THE AMOUNT AND TIMES EATEN IN THE APPROPRIATE COLUMNS.

I will ask you about the type and the amount of food the child has been eating during the last 6 months. Please tell me if the child eats the food, how much the child eats and how often the child eats it.

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
PORRIDGE	Maize-meal Porridge	Stiff (Pap)	4278	1c stiff = 250 g 1T = 75g						
		Soft (Slappap)	4277	1c soft = 250g 1T = 75g						
		Crumbly (Phutu)	4279	1 c crumbly = 140 g 1T = 30g						
	Sour Porridge	Maize with Vinegar Maize Fermented Mabella with Vinegar Mabella Fermented	P0001 P0002 P0003 P0004	½c = 125g 1c = 250g						
	Mabella Porridge/Cornrice	Stiff	3437	½ c = 125g						
		Soft	3437							
	Maltabella Porridge	Stiff	3241	½ c = 125g						
		Soft	3241							
	Oats Porridge		3239	2c = 125g						
	Nutritional scheme products e.g. Philiani Yabantwana	Stiff		4 E = 50g						
		Soft								
	Other Cooked Cereals									
	Milk on Porridge (Circle type usually used)	None								
		Whole/Fresh	2718	little = 30g med = 60g much = 125g						
		Sour	2787							
		2%	2772							
		Fat Free / Skim	2775							
		Milk Blend	2771							
		Soy Milk	2737							
		Condensed (Whole, Sweet)	2714		1t = 10g					
		Condensed (Skim, Sweet)	2744							
		Evaporated Whole	2715	1t = 3g						
		Evaporated Low Fat	2827							
Non-Dairy Creamer		2751	1t = 4g							
Is sugar added to porridge? (Circle type usually used)		None								
	White	3989	1t sugar = 6g							
	Brown	4005								
	Syrup	3988	1t honey/syrup = 15g							

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
		Honey	3984							
		Sweetener: Type	P0016							
	Is fat added to porridge? (Circle type usually used)	None								
		Animal Fat (Butter)	3479	1t marg/oll = 5g						
		Hard Margarine	3484							
		Soft Margarine (PM)	3496							
		Soft Margarine (Med)	3531							
		Sunflower Oil	3507							
		Peanut Butter	3485	1t = 12g						
BREAKFAST CEREALS	Breakfast Cereals	Specify types usually eaten		(See Manual)						
	Milk on Cereal	Specify Type		(See Manual)						
	Is sugar added to cereal?	Specify Type		(See Manual)						
	Is fat added to cereal?	Specify Type		(See Manual)						
I am now going to ask about starchy foods:										
STARCHES	Samp/Malze Rice	Samp, White	3250	1T = 55g; 1 SP = 125g;						
		Malze Rice	3250	¼ c = 125g						
		Sweetcorn Boiled	3725	1T = 25g; 1 SP = 45g; ¼ c 65g						
	Samp and Beans	Specify Ratio:	3402							
	Samp and Peanuts	Specify Ratio:	P0013	1T = 50g 1SP = 125g ¼ c = 125g						
	Rice: Specify Brands Names	White	3247	1T = 25g; 1SP = 60g;						
Brown		3315	¼ c = 65g							
	Stamped Wheat		3249	1T = 30g; 1SP = 80g; ¼ c=80g						
STARCHES	Pastas	Macaroni	3262	1T = 35g; 1SP = 70g;						
		Spaghetti Plain	3262	¼ c = 90g						
		Spaghetti and Tomato Sauce	3258	1T =45g; 1SP =80g; ¼ c=125g						
	Other: Specify									
	Do you add fat to any of these starchy foods?	Yes ____ No ____ If yes, specify types, amounts and to which food?		(See Manual)						

FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
BREADS AND SPREADS									
Now we come to bread and bread spreads:									
Bread/Bread Rolls	White	3210	Wh + Br 10mm - 30g Wh + Br 20mm - 60g						
	Brown	3211	Wh + Br 30mm - 100g ½ loaf - 400g						
	Whole Wheat	3212	Ww 10mm - 35g						
Other Breads (Specify Types)	Raisin	3214	m/s - 30g; L/s - 50g						
	Maize Meal	3278							
	Sweetcorn	3379							
	Rye	3213							
	Pumpernickel	3283							
	Other								
How many times per week does the child eat bread? _____									
Dumpling	(Depends on specific areas)		(See Manual)						
Vetkoek	(Depends on specific areas)		8 cm diam - 60g						
Provita		3235	6g						
Crackers	Cream Crackers	3230	8g						
	Refined (eg. Tuc)	3331	4g						
	Wholewheat	3391	8g						
Pizza	(Specify Toppings)		(See Manual)						
Hot Dogs	(Specify Sausage)		(See Manual)						
Hamburgers	(Specify Meat)		(See Manual)						
Are any of the following spreads on the child's bread? Fat Spreads: (Tick box)	Butter	3479	1t - 5g						
	Butro	3523							
	Animal Fat (Beef Tallow)	3494							
	Lard	3495							
	Hard Margarine	3484							
	Soft Margarine (PM)	3496							
	Soft Margarine (Med)	3531							
PeanutButter		3485	1t - 12g						
Sweet Spreads	Jam	3985	1t - 15g						
	Syrup	3988							
	Honey	3984							
Marmite/OXO	Marmite	4030	thin - 2g; med - 4g; thick=7g						
	Oxo	4029							
Paste	Fish Paste	3109	thin - 5g; med - 7g;						
	Meat Paste	2917	thick - 10g						

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HMM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
BREADS AND SPREADS	Cheese (Specify Types)	Cheddar	2722	grated: med - 10g; thick - 15g						
		Gouda	2723	cubes - 30g; slice - 8g; cheezl - 20g						
		Cottage Low-Fat Cheese	2760	med - 20g; thick - 30g						
		Cream Cheese	2725	thin - 10g; med - 20g						
		Other								
	Cheese Spreads (Specify Types)		2730	med - 12g; thick - 25g						
Other Spreads (Specify Types)										
You are being very helpful. Can I ask you about protein foods? These are: meat, beans, chicken, fish and eggs.										
CHICKEN		Boiled with skin	2926	Breast + skin - 125g						
		Boiled without skin	2963	Thigh - 80g						
		Fried in batter/crumbs	3018	Drumstick - 42g						
		Fried - not coated	2925	Foot - 30g						
		Roasted/grilled with skin	2925	Wing - 30g						
		Roasted/grilled without skin	2950							
	Chicken Bones Stew	(Specify ingredients)	P0048							
	Chicken Heads		2999							
	Chicken Stew	With Vegetables	3005	1SP - 90g;						
		With Tomato & Onion	2985	½ c - 125g						
	Chicken Feet		2997	Foot - 30g						
	Chicken Offal	Giblets	2998	stomach - 20g						
	Chicken Liver		2970	Liver - 30g						
Chicken Pie	Commercial or homemade	2954	med - 150g							
RED MEAT	Beef	Roasted with Fat	2944	120 x 60 x 5 - 35g						
		Roasted, Fat Trimmed	2960	120 x 60 x 10 - 70g						
		Rump, Fried with Fat	2908	S/s 130 x 70 x 15 - 125g						
		Rump, Fried, Fat Trimmed	2959	L/s 165 x 70 x 30 - 270g						
		Stewed/Boiled With Fat (Cabbage)	3006	1SP - 105g; ½ c - 125g						
		Stewed/Boiled Without Fat (Vegetables)	2909							
		Mince With Tomato and Onion	2987	1T-40g; 1SP-85g; ½ c-100g						
		Other Preparation Methods:								

FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
Mutton	Fried/Grilled: With Fat	2927	Loin chop - 60g;						
	Fried/Grilled: Without Fat	2934	Rib chop - 40g						
	Stew: Plain	2974	1SP - 105g;						
	Stew: Irish (Vegetables)	2916	½ c - 125g						
	Stew: Curry	3039							
	Stew: Greenbean	3040							
	Other Preparation Methods:								
Pork	Fried/Grilled: With Fat	2930	Chop: 115 x 80 x 20 - 100g						
	Fried/Grilled: Without Fat	2977	Schnitzel: 115 x 80 x 20 - 110g						
	Roast With Fat	2958	Roast: 110x 65 x 5 - 30g						
	Roast Without Fat	2978	1SP - 105g; ½ c - 125g						
	Other Preparation Methods:								
Goat	Fried/Grilled: With Fat	P0008	120 x 60 x 5 - 35g						
	Fried/Grilled: Fat Trimmed	P0009	120 x 60 x 10 - 70g						
	Stewed (Plain)	4281	1SP - 105g						
	Stewed (With Vegetables)	4282	½ c - 125g						
	Other Preparation Methods:								
MEAT: GENERAL	Offal	"Veldern" Fried	P0023	1SP - 105g; ½ c - 125g					
		Liver: Beef (Fried)	2920	80g					
		Liver: Sheep (Fried)	2955	55g					
		Kidney (Beef)	2923	85g					
		Kidney (Sheep)	2956	30g					
		Tripe, Beef, Cooked in Milk	2951	1SP - 105g; ½ c - 125g					
		Heart (Beef)	2968	60g					
		Heart (Sheep)	2969	60g					
		Lung (Beef)	3019	60g					
Wors/Sausage	Fried	2931	Thin x 200mm - 45g; Thick x 165mm - 90g						
Bacon	Fat	2906	1 rasher - 10g						
	Lean	2915							

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/ NEV
FISH	Cold Meats	Polony	2919	Slice 5mm thick - 8g Comm slice - 16g						
		Ham	2967	Med slice - 25g						
		Viennas	2936	100mm - 30g; 150mm -40g						
		Other								
	Canned Meats	Bully Beef	2940	138 x 85 x 3 - 20g; ½ c - 100g						
		Other (Specify)								
	Meat Pie	Bought (Steak & Kidney)	2957	120g						
		Other (Specify)								
	Legumes (Specify dried beans/peas/legumes)	Stews (Bean, Potato & Onion)	3178	1T=60g; 1SP = 120g; ½c=125g						
		Soups: Commercial	3165	½ c - 125g						
		Split Pea	3157	1T=35g; 1SP = 80g;						
		Lentil	3153	½ c - 130g						
		Beef & Vegetables	3159							
		Bean	3145							
		Legume Salad	3174	1T=40g; 1SP=105g; ½ c=135g						
	Soya Products e.g. Toppers / Imana	(Specify)	3196	1SP = 85g; ½ c = 120g						
	Fried Fish (Fresh or Frozen, Fried in Sun Oil)	With Batter/Crumbs	3094	Small 50 x 55 x 30 - 60g;						
		Without Batter/Crumbs	3084	Med 100 x 55 x 30 - 120g						
	Canned Fish	Pilchards In Brine	3055	1 Pilchard - 75g						
		Pilchards In Tomato Sauce	3102							
Pilchards, Mashed		3102	1 SP = 85g; ½ c = 100g							
Sardines In Oil		3104	Ss - 7g; L/s - 25g							
Sardines In Tomato Sauce		3087								
Tuna In Oil		3093	¼ c = 50g							
Tuna In Brine		3054								
Other (Specify)										
Do you remove fish bones before eating canned fish? Yes ___ No ___										

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT, USUALLY EATEN (HHM)	AMOUNT, USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
We now come to vegetables:										
VEGETABLES	Cabbage	Bolled, Nothing Added	3756	1T=30g; 1SP=55g; ½ c=80g						
		Bolled with Potato, Onion and Fat	3813	1T=35g; 1SP=75g; ½ c=80g						
		Fried, Nothing added	3812	1T=30g; 1SP=55g; ½ c=80g						
		Bolled, then fried with potato, onion	3815	1T=35g; 1SP=75g; ½ c=80g						
		Other								
	Spinach/Marog/imifino/ Amaranth Leaves Other Green Leafy Vegetables: List Names	Bolled, nothing added	3980	1T=40g; 1SP=105g; ½ c=90g						
		Bolled, fat added	3898	1T=40g; 1SP=105g; ½ c=90g						
		Bolled with Onion, Potato and Fat	3901	1T=50g; 1SP=105g; ½ c=110g						
		Bolled with Peanuts	P0015	1T=55g; 1SP=120g; ½ c=105g						
		Other:								
	Tomato and Onion "Gravy"/ Relish/Chow/Sheshebo	Home Made with Sugar	3910	1T = 35g; 1SP = 75g; ½ c = 140g						
		Home Made, no Sugar	3925							
		Canned	4192							
	Pumpkin (Specify Type)	Bolled, nothing added	4164	1T = 45g; 1SP = 85g; ½ c = 105g						
		Cooked In Fat and Sugar	3893							
		Other								
	Carrots	Bolled, Sugar and Fat	3818	1T = 25g; 1SP=60g; ½ c = 85g						
		With Potato/Onion (HM)	3822	1T=35g; 1SP=70g; 1/2 c=105g						
		Raw, Salad (Sugar added)	3721	1T = 25g						
		Other								
	Mealies/Sweet Corn	On Cob	3725	1T =30g; 1SP = 60g; ½ c =95g						
		Off Cob – Creamed, Sweet Corn	3726	1T = 55g; 1SP = 125g;						
		Off Cob – Whole Kernel Canned	3942	½ c = 135g						
		Other								
	Beetroot	Cooked (No Sugar)	3698	1T=40g; 1SP = 70g;						
(With Sugar)		3699	½ c = 80g							
Salad (Grated)		3699	1T = 25g; 1SP = 65g							

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV	
VEGETABLES	Potatoes	Boiled/Baked with Skin	4155	S/s = 60g; m/s = 90g							
		Without Skin	3737								
		Mashed (WM)	3876	1T=50g; 1SP = 115g; ½ c = 125g							
		Roasted	3878	1 med = 70g							
		French Fries/Potato Chips	3740	½ c = 50g; med = 80g							
		Salad	3928	1T = 45g; 1SP = 105g; ½ c = 120g							
		Other									
	Sweet Potatoes	Boiled/Baked with Skin	3748	1T = 50g; 1SP = 110g; ½ c = 145g							
		Without Skin	3903								
		Mashed (With Sugar)	3749								
		Other									
	VEGETABLES	Green Beans	Green, Frozen	4123	1T = 25g; 1SP=60g; 1/2 c=80g						
			Cooked, Potato & Onion (HM)	3792	1T = 40g; 1SP = 75g; ½ c = 120g						
			Other								
Peas		Green, Frozen, Boiled	4146	1T=30g; 1SP = 65g; ½ c = 65g							
		Green, Frozen with Sugar, Boiled	3720								
		With Sugar and Butter	3859								
Salad Vegetables		Raw Tomato	3750	Med = 120g; slice = 15g							
		Lettuce	3723	1 med leaf = 30g							
		Cucumber	3718	Med slice = 10g; thick = 15g							
		Avocados	3656	¼ avo (80 x 50mm) = 40g							
Other Vegetables: Specify											
VEGETABLES	If you fry vegetables or add fat, specify type of fat usually used	Butter	3479	1t = 5g							
		Butro	3523								
		Animal Fat (Beef Tallow)	3494								
		Lard	3495								
		Hard Margarine (Brick)	3484								
		Soft Margarine (Tub, PM)	3496								
		Soft Margarine (Med)	3531								
	Other										

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV	
DRESSINGS	Mayonnaise/Salad Dressing	Mayonnaise - Bought	3488	1t = 10g							
		- Home-made	3506	1T = 40g							
		Cooked Salad Dressing	3503	1t = 5g; 1T = 15g							
		Salad Dressing, low-oil	3505								
		Salad Dressing, French	3487								
		Oil - Olive Oil	3509	1t = 5g; 1T = 15g							
		- Sunflower Oil	3507								
		- Canola	4280								
FRUIT	How many times a week does the child eat vegetables? _____										
	How many times will this be fresh? _____ Canned _____ Frozen _____										
	I will now ask about fruit										
	Apples	Fresh	3532	1T=60g; ½ c = 120g;							
		Canned, Pie, Unsweetened	4216	1 med = 150g (52 x 66)							
	Bananas		3540	1 med = 75g							
	Oranges/Naartjies		3560	Med (7cm) = 180g							
	Grapes		3550	Med bunch = 230g; ½ c = 90g							
	Peaches	Fresh	3565	1 med = 150g (60 x 65)							
		Canned In Syrup	3567								
	Apricots	Fresh	3534	1 med = 35g							
		Canned In Syrup	3535								
Mangoes	Fresh	3556	135mm = 350g								
	Canned In Syrup	3633									
Pawpaw		3563	Wedge 165 x 26 x 27 = 90g								
Pineapple	Raw	3581	1 slice (85 x 10mm) = 40g								
	Canned In Syrup	3648									
Guavas	Fresh	3551	Med (6cm) = 95g								
	Canned In Syrup	3553									
Pears	Fresh	3582	1 med (80 x 65mm) = 165g								
	Canned In Syrup	3583									
Other Fruit											

FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
Dried Fruit (Also as Snacks)	Raisins	4232	1 handful = 27g						
	Prunes (Raw)	4230	1T = 50g; ½ c = 110g; 1 = 12g						
	Prunes (Cooked with Sugar)	3564							
	Peaches (Raw)	3568	1 med = 150g (60 x 65)						
	Peach (Cooked with Sugar)	3569							
	Apples (Raw)	3600	1T=60g; ½ c = 120g; 1 med = 150g (52 x 66)						
	Dried Fruit Sweets	3995		(See Manual)					
	Other								
How many times a week does the child eat fruit? _____									
How many times will this be fresh _____ Canned _____ Frozen _____									
DRINKS	Tea	Ceylon	4038	Teacup = 180ml; mug = 250ml					
		Rooibos	4054						
	Sugar Per Cup of Tea	Specify Type: White	3989	1t sugar = 6g					
		Brown	4005						
	Milk per Cup of Tea	Fresh/Long Life Whole	2718	20ml – tea in cup					
		Fresh/Long Life 2%	2772	35ml – tea in mug					
		Goat	2738	40ml – coffee in cup					
		Fresh/Long Life from (skimmed)	2775	75ml – coffee in mug					
	Whole Milk Powder Reconstituted (Specify Brand)	2831	1t = 4g						
	Skimmed Milk Powder, reconstituted (Specify Brand)	2719	1t = 4g						
	Milk Blend, reconstituted (Specify Brand)	2771	20ml – tea in cup 35ml – tea in mug 40ml – coffee in cup 75ml – coffee in mug						
	Whitener/non-dairy creamer (Specify Brand)	2751	1t = 4g						
	Condensed Milk (Whole)	2714	1t = 10g						
	Condensed Milk (Skim)	2744							
	Evaporated Milk (Whole)	2715	1t = 3g						
	Evaporated Milk (Low-Fat)	2827							
	None								
Coffee		4037	Teacup = 180ml; mug = 250ml						

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HMM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
	DRINKS	Sugar per Cup of Coffee	Specify Type: White Brown	3989 4005	1t sugar = 6g					
Milk per Cup of Coffee		Specify Type		(See Manual)						
Milk as such: What type of milk does the child drink as such?		Fresh/Long Life/ Whole	2718	To drink 1/2 c = 125ml						
		Fresh/Long Life/2%	2772	Baby bottle = 250ml						
		Fresh/Long Life/Fat Free (skimmed)	2775							
		Goat	2738							
		Sour/Maas	2787							
Milk drinks. Specify Brands, including milk supplements and type of milk used		Nestle Drinking Chocolate	4287	1t = 5g						
		Malted Milk Beverage, no Sugar (eg Milo)	2735	1t = 5g						
		Flavoured Milk	2774	Carton = 250ml; S/s plastic = 350 ml						
	Other									
	Nutritional scheme products e.g Nutrimil Junior.									
DRINKS	Yoghurt	Drinking Yoghurt	2756	S/s = 175ml						
		Thick Yoghurt: Plain, Fat-Free	2778	Yogisip = 350ml						
		WM Plain	2757	1/2 c = 125g						
		Fruit, Low Fat	2732							
		Other								
	Squash	Sweeto, Sixo	3982	Small glass = 150ml Medium glass = 250 ml						
		Oros/Lecol with Sugar	3982	Large glass = 500 ml						
		Artificial Sweetener	3990	S/s bottle = 350ml L/s bottle = 500ml						
		Kool Aid	3982	S/s can = 350ml						
		Other								
	Fruit Juice	Fresh/Liquifruit/Ceres/Purty	2866	1 Liquifruit s/s = 250ml						
		"Tropica"/mixture with milk	2791	1 Liquifruit L/s = 500 ml S/s bottle = 350ml L/s bottle = 500ml S/s can = 350ml						
	Fruit Syrups	Average	2865	1t = 5g						
		Guava Syrup	2864							
	Fizzy Drinks (e.g. Coke, Fanta)	Sweetened	3981	S/s bottle = 350ml L/s bottle = 500ml						
		Diet	3990	S/s can = 340ml						

FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	DW	P/M	SEL/NEV
Magou/Motogo		4056	1 carton = 500 ml						
Other (Please Specify)									
SNACKS									
Please indicate what types and amounts of snacks, puddings and sweets the child eat:									
Potato Crisps		3417	(See Manual)						
Peanuts	Roasted Unsalted	3452							
	Roasted, Salted	3458							
Cheese Curis (Nik Naks, etc.)	Average	3267							
	Savoury	3418							
Popcorn	Plain	3332							
	Sugar Coated	3359							
Peanuts and Raisins (mixed)	Roasted, Salted	P0047							
Chocolates	Specify types and names: Assorted	3992		(See Manual)					
Candies	Sugus, gums, hard sweets (Specify)	3986							
Sweets	Toffee, fudge, caramels (Specify)	3991							
How many times a week does the child eat snack food? _____									
CAKES, BISCUITS AND COOKIES									
Biscuits/Cookies	Specify Type		(See Manual)						
Cakes & Tarts	Specify Type								
Pancakes/Crumpets	Specify Type								
Rusks	Specify Types								
Scones	White, WM	3237	6cm diam=35g;						
Muffins	Plain	3408	8cm diam=60g						
	Bran	3407							
Koekalsters		3231	100 x 35 = 60g						
Savouries	Sausage Rolls	2939	Roll x 135mm = 165g						
	Samosas (Meat)	3355	S/s = 42g						
	Biscuits e.g. Bacon Klips	3331	4g						
	Other								

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV	
	How many times a week does the child eat cakes/cookies? _____ less than 1/week _____										
PUDDINGS	Jelly		3983	1T=35g; 1SP=75g; ½ c = 110g							
	Baked Puddings	Specify Types		Med serving = 30g 30 x 65 x 65 = 50g							
	Instant Puddings	Specify Types		1T = 45g; SP = 95g; ½ c = 145g							
	Ice Cream	Commercial Regular		3483	Scoop = 40g; 1SP=65g;						
		Commercial Rich		3519	½ c = 75g						
		Soft serve		3518	Plain = 135g; + flake = 155g						
		Sorbet		3491	Scoop = 40g; 1SP=65g;						
		Ice Lollies		3982	½ c = 75g						
		Chocolate Coated Individual Ice Creams (E.g. Magnum)		P0036							
	Custard	Home Made (WM)		2716	T=13g; SP = 40g						
(SM)			2717								
Other Puddings											
	Specify										
	How many times a week does the child eat pudding? _____ less than 1/week _____										
SAUCES, GRAVIES, COMPLEMENTS	Tomato Sauce		3139	1t = 6g; 1T = 25g							
	Worcester Sauce		P0037								
	Chutney	Fruit		3168	1t = 14g; 1T = 60g						
		Tomato		3114							
	Others										

1. EATING PATTERNS: (FREQUENCY OF EATING)	
Please indicate which of the following best describes the eating pattern the child usually follows (mark only one)	
More than three meals with eating between meals	1
Three meals with eating between meals	2
Three meals with no eating between meals	3
Two meals with eating between meals	4
Two meals with no eating between meals	5
One meal with eating between meals	6
One meal with no eating between meals	7
Nibble the whole day, no specific meals	8
Others (Please specify):	9

2. Are there any foods that the child eats which we haven't talked about? Please list them.							
FOODS	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE
			Per day	Per week	Per month	Seldom/ Never	

Thank you for your co-operation. We appreciate your contribution.

ABBREVIATIONS:

<u>Measures</u> 1t = 1 rounded teaspoon 1T = 1 rounded tablespoon (15ml) 1SP = 1 rounded servingspoon (30ml) c = measuring cup (250ml) s/s = small size m/s medium L/s = large E = enriched P = plain <u>Milk:</u> SM = skim milk WM = whole milk BL = blend CON = condensed	<u>Bread:</u> Wh = white Br = brown Ww = wholewheat <u>Meat:</u> F = with fat FT = fat trimmed <u>Oil/Fat</u> B = butter HM = hard margarine Med = medium fat/light PM = polyunsaturated SO = sunflower oil WF = white fat PB = peanut butter	BR = breakfast (Up to 09h00) IS = in-between snack L = lunch (midday (12h00-14h00) D = dinner (evening) (17h00 - 19h00) AD = after dinner Comm = commercial Home = homemade Pot = potato Cab = cabbage Carr = carrot Fill = filling Usually = at least 4x/week <u>Other</u> HHM = Household Measure P/D = Per day D/W = Days Per Week P/M = Per Month SEL/NEV = Seldom / Never
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Addendum 6: Standard operating procedure for collection of blood samples

Blood will be drawn by a Paediatric Phlebotomist

1. Phlebotomist will draw 7 millilitres (mls) of blood and put:

- 4 mls in Red top test tubes (No additive in the test tube)

2 During the day all the test tubes containing blood will be stored in a cooled Ice box and Red top and Purple tops test tubes will be taken to the Hematology department laboratory at the Tygerberg Academic Hospital.

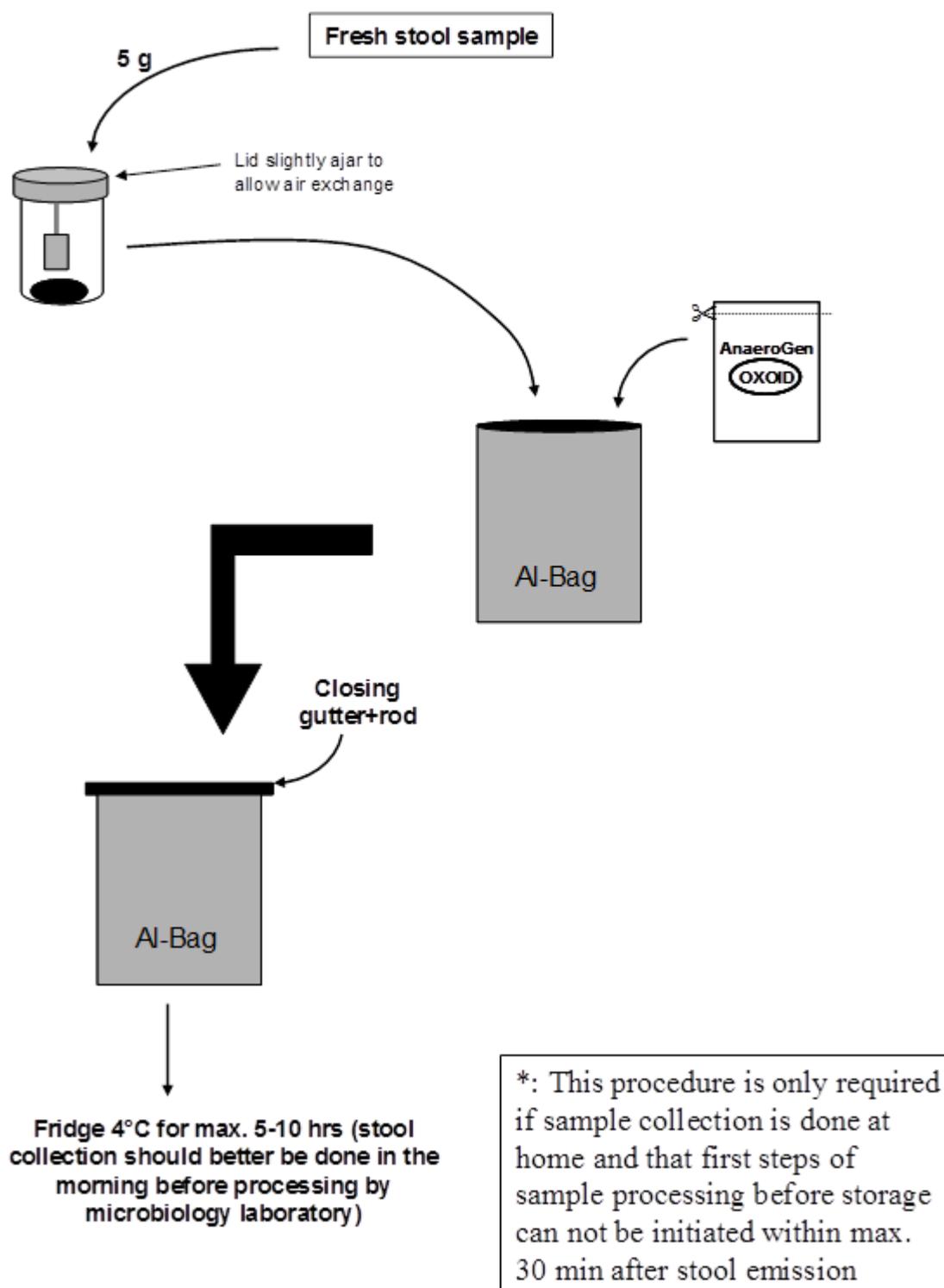
The following tests will be performed on the blood in the Red top test tubes:

- C- reactive protein

Addendum 7: Information sheet for caregiver to collect faecal sample from child

Flowchart for Stool Sampling*

Classical microbial analysis



Procedure for stool collection

Sample for classical microbial analysis of the stool

Routine stool collection at home:

1. Fresh stool must be collected and prepared according to the following steps (2-8) within a maximum of 30 min after stool emission.
2. Transfer 5 g (equivalent to a rounded spoonful) into a sterile tube with a spoon attached in the cap.
3. The lid of the container is left slightly ajar to allow air exchange.
4. The container is identified with the study number, subject number, and time point (i.e. date of collection).
5. The container is placed in the aluminium bag.
6. A packet of AnaeroGen (Oxoid) is cut open along the top and placed inside the bag in an upright position.
7. The bag is closed tightly with a plastic closure device. The plastic rod is positioned just below the top of the aluminium bag. Bag and rod are clipped together using the plastic gutter.
8. The bag is kept refrigerated (4°C) and transported on ice as quickly as possible to the microbiology laboratory (maximum 5-10 hours after stool emission, stool should be better collected in the morning).

Addendum 8: Procedure for fecal processing

Sample for classical protein analyses (approx. 5 g containing tube received at the clinic):

1. Approximately. 0.5 g of stool is transferred in a 2 ml Eppendorf tube and homogenized into protein extraction buffer according to the procedure described in the protocol before storage at -20°C (for proteins and sIgA analyses).

Addendum 9: Definition of an Adverse event

Adverse Event documentation

Instructions for “Adverse Events”

Definition: Adverse event

An adverse event is defined as any untoward occurrence in a patient or clinical investigation study participant administered an investigational product and which does not necessarily have to have a causal relationship with this treatment.

Adverse events are illnesses, signs or symptoms (including an abnormal laboratory finding) occurring or worsening in the course of the study. Adverse events can be serious or minor. They may or may not lead to the withdrawal of the study participant/patient from the study. All adverse events must be documented and assessed for relationship to the study product.

Investigators must know and record the following information about adverse events:

- Study participant and date
- Description of event
- Duration
- Frequency
- Intensity
- Seriousness
- Action taken
- Outcome and sequelae
- Relationship to test product

Intensity

Mild: symptoms hardly perceived, only slight impairment of general well-being.

Moderate: clearly noticeable symptom, but tolerable without immediate relief.

Severe: overwhelming discomfort.

Seriousness

Serious: a serious adverse event is a fatal or life threatening event causing permanent harm or requiring / extending in-patient treatment at a hospital or which is considered medically relevant by the physician.

Non-serious: all other adverse events not corresponding to the definition of serious adverse event, are considered as non-serious.

Relation with test product

The study physician will assess the possibility of a link between the study product and an adverse event on the basis of the following criteria:

- Unrelated: There is an **evident** other explanation for the AE, e.g.,
- The AE is obviously explained by the patient's disease
 - The AE is in accordance with the effect or adverse effect of the concomitant medication
 - The AE has occurred already prior to the administration of the study product
- Unlikely relation: Reasonable temporal relationship with the intake of the study product, **but**
- There is another plausible explanation for the occurrence of the AE.
- Probable relation: Reasonable temporal relationship with the intake of the study product **and**
- Plausible reasons point to a causal relationship with the study product
- Certain relation: Reasonable temporal relationship with the intake of the study product **and**
- There is no other explanation for the AE **and**
 - Subsidence or disappearance of the AE on withdrawal of the study product (de-challenge) **and**
 - Recurrence of the symptoms on re-challenge

During a severe infection process, we recommend the use of antibiotics for which the probiotics in Nutren Jr Plus are highly sensitive: Penicillin-streptomycin, erythromycin and ciprofloxacin. In addition, samples should be collected and stored for further analysis including the probiotic strains.

Reporting and Documentation

Serious adverse event

Project management and CRA/monitor must be notified of all serious or unexpected adverse events within 48 hours per fax. Notification does not depend on whether there is a connection to the study formula or not.

Please fax SAE form to:

Edwardo Schiffrin, M.D.

Nestle Nutrition

Tel: +41 21 924 4730

Fax: +41 21 924 4527

Non-serious adverse event

All adverse events must be documented on the appropriate pages of the case report forms (AE).

Follow up

In the case of a serious adverse event(s) persisting beyond trial termination, a follow up visit may be required. Further, in the event that further analyses are required for the evaluation of a potential cause-effect relationship between the study product and the adverse event, all examinations and laboratory analyses and their results will be documented in the case report forms

	form (section SAE) and fax it to Sponsor	form (section SAE) and fax it to Sponsor	form (section SAE) and fax it to Sponsor
3. Date of first appearance	_ _ _ _ _ _ _ _ Day Month Year	_ _ _ _ _ _ _ _ Day Month Year	_ _ _ _ _ _ _ _ Day Month Year
Duration	_ _ _ _ Hours Days	_ _ _ _ Hours Days	_ _ _ _ Hours Days
Frequency	Once <input type="checkbox"/> 4. Several times <input type="checkbox"/> Ongoing <input type="checkbox"/>	Once <input type="checkbox"/> 5. Several times <input type="checkbox"/> Ongoing <input type="checkbox"/>	Once <input type="checkbox"/> 6. Several times <input type="checkbox"/> Ongoing <input type="checkbox"/>
Intensity	Mild (1) <input type="checkbox"/> Moderate (2) <input type="checkbox"/> Severe (3) <input type="checkbox"/>	Mild (1) <input type="checkbox"/> Moderate (2) <input type="checkbox"/> Severe (3) <input type="checkbox"/>	Mild (1) <input type="checkbox"/> Moderate (2) <input type="checkbox"/> Severe (3) <input type="checkbox"/>
Did the AE lead to discontinuation of the study?	Yes <input type="checkbox"/> No <input type="checkbox"/> 7. If yes: fill in section CD compl./discont. of study	Yes <input type="checkbox"/> No <input type="checkbox"/> 8. If yes: fill in section CD compl./discont. of study	Yes <input type="checkbox"/> No <input type="checkbox"/> 9. If yes: fill in section CD compl./discont. of study
	<i>(Continued next page)</i>	<i>(Continued next page)</i>	<i>(Continued next page)</i>

(Adverse Event 1-3 Page 2/2)

	<i>(Continued)</i>	<i>(Continued)</i>	<i>(Continued)</i>
	Adverse Event 1	Adverse Event 2	Adverse Event 3
Did the AE lead to the discontinuation of the trial product?	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>
If yes:	yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>
AE disappeared?	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>
Trial product re-introduced?	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>
If yes: Reappearance of AE?			
Date of last occurrence:	_ _ _ _ _ _ _ _ _ _ _ Day Month Year	_ _ _ _ _ _ _ _ _ _ _ Day Month Year	_ _ _ _ _ _ _ _ _ _ _ Day Month Year
10. Outcome:	AE disappeared <input type="checkbox"/> AE ongoing <input type="checkbox"/> unknown <input type="checkbox"/>	AE disappeared <input type="checkbox"/> AE ongoing <input type="checkbox"/> Unknown <input type="checkbox"/>	AE disappeared <input type="checkbox"/> AE ongoing <input type="checkbox"/> Unknown <input type="checkbox"/>
Had the patient experienced the	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>

AE before, (without trial product)?	Unknown <input type="checkbox"/>	Unknown <input type="checkbox"/>	Unknown <input type="checkbox"/>
Treatment of the AE:	Yes <input type="checkbox"/> No <input type="checkbox"/> Specify:	Yes <input type="checkbox"/> No <input type="checkbox"/> Specify:	Yes <input type="checkbox"/> No <input type="checkbox"/> Specify:
Causality assessment (relation AE - trial product)	Unrelated <input type="checkbox"/> Unlikely <input type="checkbox"/> Probable <input type="checkbox"/> Certain <input type="checkbox"/>	Unrelated <input type="checkbox"/> Unlikely <input type="checkbox"/> Probable <input type="checkbox"/> Certain <input type="checkbox"/>	Unrelated <input type="checkbox"/> Unlikely <input type="checkbox"/> Probable <input type="checkbox"/> Certain <input type="checkbox"/>
Comments:			
Signature:			

Form For Registration of Serious Adverse Event #1 (SAE)

(Page 1 of 2)

Date of birth: |_|_|_|_|_|_|_|_|_|_| Current weight (kg): |_|_|_| . |_| Sex: M F

Subject data	day month year
	Date of 1 st study product administration: _ _ _ _ _ _ _ _ _ _
	day month year
Date of last administration before onset of SAE: _ _ _ _ _ _ _ _ _ _ Time: _ _ _	
: _ _ _	

day month year hrs
mins

Last dose/amount received before onset of SAE (ml/kg/day): |_|_|_|_|_| or not applicable

Information about the event	Date of onset of SAE: _ _ _ _ _ _ _ _ _ _	Time at onset: _ _ _ : _ _ _
	day month year	hrs mins
	Description of event: _____ _____ _____	
	Time between onset of SAE and last administration of study formula: _ _ _ : _ _ _	
hrs mins		
Duration of symptom: _ _ _ : _ _ _		
hrs mins		

Date of resolution: |_|_|_|_|_|_|_|_|

Time of resolution:

|_|_| : |_|_|

or continuing

day month year

hrs mins

Differential diagnosis considered: _____

Seriousness	Clinical course	Causal relationship (investigator's opinion)
Subject died <input type="checkbox"/>	Persistent event <input type="checkbox"/>	Unrelated <input type="checkbox"/>
Hospitalisation needed/prolonged <input type="checkbox"/>	Improving <input type="checkbox"/>	Unlikely <input type="checkbox"/>
	Recovered <input type="checkbox"/>	Probable <input type="checkbox"/>
Persistent or significant disability <input type="checkbox"/>	Recovered with sequelae <input type="checkbox"/>	Certain <input type="checkbox"/>
Congenital anomaly/birth defect <input type="checkbox"/>	Worsening <input type="checkbox"/>	
Medically relevant event <input type="checkbox"/>	Death <input type="checkbox"/>	
	Unknown <input type="checkbox"/>	

Previous adverse reaction to similar treatment: Yes No

Unknown

If yes, give details: _____

Measures taken concerning the study product:

None Withdrawal of study product → Date of withdrawal: |_|_|_|_|_|_|_|_|

day month year

Dose reduction specify: _____

Reintroduced and positive Reintroduced and negative

Other, specify: _____ (*continued next page*) →

SAE #1 Report (Page 2 of 2)

Corrective therapy: _____

Code breaking: Yes No Not applicable

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If yes, product code or name: _____ Batch No:

Concomitant medication:

Name	Indication	Route of administration	Dose (units)	Dates	
				Started	Ended

If further examinations are required to assess causality, note findings and attach results of examinations (lab reports, X-rays, etc): _____

Date of last administration before onset of SAE: |_|_|_|_|_|_|_|_|_|_| Time: |_|_|_|
 : |_|_|_|

day month year hrs
 mins

Last dose/amount received before onset of SAE (ml/kg/day): |_|_|_|_|_| or not applicable

Information about the event

Date of onset of SAE: |_|_|_|_|_|_|_|_|_|_| Time at onset: |_|_|_|_| : |_|_|_|_|
 day month year hrs mins

Description of event: _____

Time between onset of SAE and last administration of study formula: |_|_|_|_| : |_|_|_|_|
 hrs mins

Duration of symptom: |_|_|_|_| : |_|_|_|_|
 hrs mins

Date of resolution: |_|_|_|_|_|_|_|_|_|_| Time of resolution:
 |_|_|_|_| : |_|_|_|_| or continuing
 day month year hrs mins

Differential diagnosis considered: _____

Seriousness	Clinical course	Causal relationship
--------------------	------------------------	----------------------------

		(investigator's opinion)
Subject died <input type="checkbox"/>	Persistent event <input type="checkbox"/>	Unrelated <input type="checkbox"/>
Hospitalisation needed/prolonged <input type="checkbox"/>	Improving <input type="checkbox"/>	Unlikely <input type="checkbox"/>
	Recovered <input type="checkbox"/>	Probable <input type="checkbox"/>
Persistent or significant disability <input type="checkbox"/>	Recovered with sequelae <input type="checkbox"/>	Certain <input type="checkbox"/>
Congenital anomaly/birth defect <input type="checkbox"/>	Worsening <input type="checkbox"/>	
Medically relevant event <input type="checkbox"/>	Death <input type="checkbox"/>	
	Unknown <input type="checkbox"/>	

Previous adverse reaction to similar treatment: Yes No
 Unknown

If yes, give details: _____

Measures taken concerning the study product:

None Withdrawal of study product → date of withdrawal: |_|_|_|_|_|_|_|_|
 day month year

Dose reduction specify: _____

Reintroduced and positive Reintroduced and negative

Other, specify: _____ *(continued next page)*

SAE #2 Report (Page 2 of 2)

Corrective therapy: _____

Code breaking: Yes No Not applicable

If yes, product code or name: _____	Batch	No:
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Concomitant medication:

Name	Indication	Route of administration	Dose (units)	Dates	
				Started	Ended

If further examinations are required to assess causality, note findings and attach results of examinations (lab reports, X-rays, etc): _____

Other medical comments: _____

Principal Investigator (or Co-Investigator):

Printed name

Signature

Date

Please fax this SAE form within 48 hours of onset of event (pages 1 and 2) to:

Nestec (Edwardo Schiffrin, M.D, fax +41 21 21 924 4527)

