DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH,UNIVERSITY OF KWAZULU-NATAL

Effect of infant feeding mode and maternal nutritional supplementation on the nutrition and health of HIV positive mothers and their infants

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Prof Anna Coutsoudis

Supervisor

Dedication

To the efforts of all who have worked tirelessly to improve the quality of life of adults and children affected by HIV/AIDS

And

To my parents who have always encouraged and inspired me to learn

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Table of contents **Page**

List of figures

List of Tables Page

Preface

"Our message today is one of hope. It is about potential waiting to be fulfilled: the surest way to meet the global challenges we face now and in the future is to "make every mother and child count".

Dr Lee Jong-Wook, Former WHO Secretary-General *7th April 2005*

Living in any developing country, you are faced with the reality that maternal well-being is the cornerstone not only for child survival but also for the family entity. The Millennium development goals 1 through 6 all need survival and empowerment of women to see any success by 2015. According to the WHO, sub-Saharan Africa bears the largest burden (91 %) of the global population of pregnant women living with HIV requiring antiretrovirals for prevention of mother to child transmission (PMTCT). This study was undertaken to help understand the effects of breastfeeding on maternal and child health and to examine if a nutritional intervention could help attenuate any metabolic demands on an HIV infected lactating mother thereby improving maternal and child outcome while preserving exclusive breastfeeding.

Abstract

Background: Breastfeeding is known to have benefits both for maternal and child health. Some questions around the benefits and risks of breastfeeding in the presence of HIV infection still remain unclear.

Aims: To study the effects of infant feeding mode by HIV-positive mothers, on maternal and child health. In addition, to assess the effect of nutritional supplementation to HIV-positive lactating mothers on nutritional and health status of mothers and their infants and on the quality of breastmilk.

Methods: The study had 2 components; a prospective study to examine the impact of infant feeding mode on nutritional and health indices in mothers and their infants and within it a nested randomized controlled clinical trial to study the impact of a daily 50 g soya/peanut based supplement during breastfeeding on the above parameters. The measurements included anthropometry; body composition indicators (using both deuterium dilution and BIA); haematology and biochemical markers; as well as incidence rates of opportunistic infections and clinical disease progression. Breastmilk was analysed for both macro and micronutrients. Cervical screening was offered to all the women.

Results: AFASS criteria were fulfilled by 38.7% of the formula feeding mothers. No significant differences between the formula feeding and breastfeeding groups in terms of haematological, immunological and body composition changes were seen. Breastfeeding mothers had significantly lower events with high depression scores ($p=0.043$). Longer duration of breastfeeding was observed to be significantly associated with a mean increase in CD4 count (74 cells/μL) and better health outcomes. The supplement made no significant impact on any maternal or child outcomes except for a limited effect on mothers with low BMI, where it was significantly associated with preventing loss

of lean body mass (p=0.026). Breastfeeding infants had a significantly lower risk of diarrhoea and hospitalisation at 3 months (p=0.006 and 0.014 respectively). Both breastfeeding and longer duration of breastfeeding was significantly associated with better development scores and growth parameters. Supplementation made no impact on breastmilk composition. Of the 86 mothers who agreed for cervical screening, 27.6% had human papilloma virus infection.

Conclusions: Breastfeeding is not harmful to the mother despite the presence of HIV infection. On the contrary we observed both breastfeeding and longer breastfeeding duration to be associated with better maternal and child outcomes. Mothers are still choosing formula feeding inappropriately presumably because of the availability of free formula and/or sub-optimal counseling. The new (2010) local PMTCT guidelines based on WHO recommendations should reverse this. Food insecurity was prevalent amongst 32% of our study population, highlighting the need to include sustainable and empowering solutions to encounter this problem. Less sustainable solutions such as nutritional supplementation should be targeted to the malnourished and in emergency situations.

Keywords (for database): HIV positive mothers, HIV exposed infants, vertical transmission, breastfeeding, micronutrients, lean body mass, nutrition supplement, body composition, anthropometry, SRQ 20, WHO stage, AFASS criteria, BIA, FTIR

List of Abbreviations

95% CI	95% Confidence intervals
AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
ARV	antiretroviral
AZT	zidovudine
BF%	body fat percentage
BIA	bioelectrical impedance analysis
BMI	body mass index
CBV	combivir contains zidovudine and lamivudine
CDC	Centers for Disease Control and Prevention, Atlanta, USA
DNA	deoxyribonucleic acid
FFM	fat free mass
FM	fat mass
HIV	human immunodeficiency virus type 1 (in this thesis HIV has been used synonymously with HIV-1)
IRR	Incidence rate ratio
ISAK	The international society for the advancement of kinanthropometry
LBM	lean body mass
LRTI	lower respiratory tract infection
LSCS	lower segment caesarian section
MTCT	mother to child transmission
MUAC	mid-upper arm circumference
NCDs	non-communicable diseases

NHLS	National Health Laboratory Services
NVP	nevirapine
PCR	polymerase chain reaction
PID	pelvic inflammatory disease
PMTCT	prevention of mother to child transmission
SANAS	South African National Accreditation System
SRQ 20	WHO validated self reporting questionnaire of 20 questions to assess mental status
TB	tuberculosis
TBW	total body water
UNAIDS	the Joint United Nations Programme on HIV/AIDS
UNICEF	United Nations International Children's Emergency Fund/ also called United Nations Children's Fund.
WHO	World Health Organization

List of Definitions

Antiretroviral (ARV): An antiretroviral is a medication which can be taken to prevent or suppress viral replication.

Antiretroviral therapy (ART): Antiretroviral therapy, also known as potent combination antiretroviral therapy, refers to the use of a combination of a minimum of three antiretroviral drugs, to control replication of the human immunodeficiency virus and halt disease progression. Regimens usually consist of antiretroviral drugs from more than one class and commonly include one non-nucleoside reverse transcriptase inhibitor (NNRTI) with two nucleoside reverse transcriptase inhibitors (NRTIs) or a protease inhibitor (PI) with two NRTIs (WHO, 2002).

Bioelectrical impedance analysis (BIA): A technique for measuring body composition which uses a battery operated unit, connected to the body by electrodes, to pass a small electric current through the body. A measurement of the impedance to the current in the body is then obtained and is used to estimate total body water and calculate fat-free mass and body fat using regression equations.

Body mass index (BMI): An index, calculated by dividing body weight in kilograms by the height squared in metres (weight/height^2), used to categorise individuals as underweight, normal, overweight and obese.

CD4 count: CD4 stands for cluster of differentiation 4. It is a type of protein present on the surface of T helper cells, regulatory T cells, monocytes, macrophages and dendritic cells. CD4 count or T cell count stands for the number of T helper lymphocytes carrying the CD4 protein per cubic millimeter /micro litre of blood. It is used as a prognostic marker in HIV infection.

Exclusive breastfeeding: When breastfeeding is the only source of nutrition without the addition of any other liquids or solids. WHO has relaxed the definition to allow administration of oral rehydration salt solution and prescribed medication.

Fat free mass (FFM)/ Lean body mass (LBM): The FFM of the body includes the muscle, bone and water as well as any other parts of the body that do not contain any fat or lipid.

Fat mass (FM): The FM includes all the fat and lipid found in the body, both the essential and non-essential

Food security: The World Food Summit of 1996 defined food security as existing "when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life".

ISAK Methods: The international society for the advancement of kinanthropometry

Karnofsky Score: This is a performance score used globally as an indicator of health and well-being and recommended for use in adults with HIV infection by WHO (2002).

PCR: It is a molecular biological technique used to amplify a single or more copies of DNA to generate multiple copies of a specific DNA sequence.

WHO staging: It is a standardised clinical staging system developed and validated by the WHO for use in HIV/AIDS. It uses simple case definitions of clinical events to define each stage (WHO, 2007b).

Chapter 1

Introduction and Literature review

Breastfeeding is the optimal source of nutrition for all infants (Hoddinott et al 2008; Walker, 2010). However, since the advent of the HIV/AIDS pandemic, there has been intense debate surrounding the subject of breastfeeding by an HIV infected mother causing confused and mixed messages in the media filtering down to the mothers. In developed countries, health care professionals were advised to contraindicate breastfeeding in the presence of HIV infection due to the risk of HIV transmission through breastmilk (CDC, 1985). The UN agencies issued a joint statement advising formula feeding in the presence of HIV infection in countries with a low risk of infectious diseases. In countries with a high risk of infant mortality due to infectious diseases and malnutrition, they advised breastfeeding regardless of HIV status (WHO, 1987).

Vertical transmission of HIV infection in the infants can be caused by intra-uterine, intra-partum or late post natal infection through breastfeeding (de Cock et al, 2000). According to the WHO (2009b) the risk of HIV transmission in the absence of any intervention in a non breastfeeding population is around 25%; and that in a breastfeeding population about 35%. Developed countries have since managed to almost completely obliterate vertical transmission using a package of interventions including caesarian sections, antiretroviral (ARV) prophylaxis and avoidance of breastfeeding (Mofenson, 2003; European Collaborative study, 2005; Naver, 2006). However, this was not feasible in cash-strapped and resource poor countries, which had been struck by a double burden of malnutrition and HIV besides the endemic risk of infectious diseases and the impact of climate change looming over their heads. It is a known fact that breastfeeding in such an environment besides being nutritive and

sustainable, also protects infants from infectious disease morbidity and mortality (Huffman and Combest, 1990; WHO, 2000a; Jones et al, 2003; Bahl et al, 2005).

In a commentary on breastfeeding, the ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology, and Nutrition) group states that there is a dire need to re-emphasize the importance and significance of breastfeeding in view of all the evidence (2009).

Early studies showed that exclusive breastfeeding for three months or more had no greater risk of vertical transmission through breastfeeding compared to formula feeding (Coutsoudis et al, 2001; Iliff et al, 2005). Although these studies had not been designed to study the effect of the type of breastfeeding on HIV transmission, these significant findings started a new debate on the type of breastfeeding- exclusive or mixed. Revised combined guidelines were issued by the inter agency task team (WHO, 2000b) stating that replacement feeding should be avoided unless it was affordable, feasible, accessible, sustainable and safe (AFASS criteria). This was reiterated in a joint statement by WHO/UNICEF (2004). The CDC (Centers of Disease Control) and European guidelines, resulted in a spillover effect in the developing countries, there was evidence that the promotion of replacement feeding was increasing use of formula feeds even amongst the HIV negative population. This became overwhelmingly clear during a CDC epidemiological investigation of a diarrhoeal outbreak in Botswana which revealed large numbers of children born to HIV negative mothers who were not being breastfed (Creek et al, 2010).

Several African studies conducted in Zimbabwe, South Africa, Malawi, Zambia and Cote d'Ivoire (Iliff et al, 2005; Coovadia et al, 2007; Becquet et al, 2007; Kuhn et al, 2008; Kilewo

et al, 2009; Peltier et al, 2009; Thior et al 2006) reported increased morbidity and mortality in infants who had either never been breastfed or had early breastfeeding cessation. In addition, the 2006 CDC investigation discussed above reported significant risk for diarrhoeal mortality associated with non breastfeeding in both HIV exposed and unexposed infants in Botswana (Creek et al, 2010). These findings were instrumental in WHO (2007a) amending its recommendations for HIV and Infant Feeding to read "Exclusive breastfeeding is recommended for HIV-infected mothers for the first six months of life unless replacement feeding is acceptable, feasible, affordable, sustainable and safe for them and their infants before that time".

Two important fields of research resulted once again in the formulation of the new WHO HIV and Infant Guidelines in 2010. These were firstly: evidence of the efficacy of antiretroviral prophylaxis (given either to the mother or infant) to reduce HIV transmission (Thior et al, 2006; Kilewo et al, 2009; Marazzi et al, 2009; Peltier et al, 2009;) and secondly evidence of the morbidity and mortality associated with either not breastfeeding or early cessation of breastfeeding on the overall HIV-free survival of infants (Rollins et al, 2008; Kagaayi et al, 2008; Horvath 2009; Kuhn et al, 2009; Marazzi et al, 2009, 2010; Homsy et al, 2010).

The underlying principle of the guidelines was to "recommend interventions while maximizing effectiveness of reducing vertical transmission, minimizing side effects for both mothers and infants while preserving future options for HIV care and treatment" (WHO, 2009b). The recommendations include WHO staging and CD4 testing antenatally for all HIV positive mothers to determine the treatment option best suited for her health: antenatal prophylaxis (if CD4> 350 cells/µL); or full antiretroviral therapy (ART) to be continued post-delivery for life (if CD4≤ 350; WHO stage 3 or 4 regardless of CD4). Feeding advice was left

to the national authorities to decide; where breastfeeding is considered the best option; exclusive breastfeeding for six months followed by introduction of complementary foods and cessation of breastfeeding by 12 months is advised; if mother was not on ART, ARV prophylaxis (Nevirapine) to be taken by the infant until one week post cessation of breastfeeding. If the mother is on ART, then the infant is to stop the prophylaxis by six weeks of age (WHO 2009b, 2010a).

1.1 Background information on HIV in South Africa

33.4 million People are living with HIV/AIDS worldwide of which 15.7 million are women aged 15 years and above. Of these 15.7 million women, an estimated 12 million are living in sub-Saharan Africa alone (WHO/UNAIDS/UNICEF, 2010). This highlights the need to have sustainable and safe measures of preventing mother to child transmission of HIV (PMTCT) while maintaining breastfeeding. A mid-year release by Statistics South Africa (Stats SA, 2010), states that about 5.24 million people are living with HIV in South Africa, with an estimated 410,000 new infections in 2010 of which 40,000 were in children. Women of reproductive age carry the greatest burden with an estimated 28 % of women attending antenatal clinics in South Africa being HIV positive (National Department of Health, 2008). KwaZulu-Natal, the second most populous province of South Africa has one of the highest antenatal prevalence rates of HIV; between 30- 42 % (National Department of Health, 2008).

The HIV/AIDS pandemic has caused havoc on an already crippled South African family structure which was still struggling in the aftermath of Apartheid. Most households are headed by single mothers or grandmothers who use their meager pension to support an extended family. Poverty and high rates of unemployment (24.5%) are only making the spread easier. In a recent community survey, it was found that in provinces like Eastern Cape

and KwaZulu-Natal only 65.5-71.5% people have access to electricity; 70.4-79.4% access to piped water; and 11.7-25.2% people have no access to any toilet facilities, well below the national average (Stats SA, 2007). The irony of the matter is that the same socio-economic factors which cause some households to not have access to basic amenities also put them at a higher risk for acquiring HIV (Humphrey, 2010). Although infecting both sexes equally, the effect of HIV/AIDS is not gender neutral. Social scientists relate the high HIV prevalence in girls aged 15-29 years, to increased vulnerability due to poor family structure and social support system; often leading to an increased tendency for transactional sex. It is a vicious cycle fueled by poverty and gender inequity (DeWaal and Whiteside, 2002).

Although far from optimal, there have been considerable strides made in the way of treatment (ART) and prevention of mother to child transmission of HIV by 2010; of the estimated 210,000 pregnant women eligible for ART, between 66-95% have received ARV prophylaxis to prevent PMTCT in South Africa, however the coverage for infant prophylaxis still remains much below the mother's at 56% (WHO/UNAIDS/UNICEF, 2010). The other worrying health statistic is that both the under- 5 mortality and the maternal mortality rates are still high at 67 per 1000 live births and 124 per 100,000 live births respectively (WHO, 2010b).

1.2 Impact of HIV on nutritional status of women

Disease manifestation, whether mild or severe, depends on the underlying nutritional and immune status of an individual. Nutrition and immunity have a synergistic relationship (Scrimshaw and SanGiovanni, 1997; Keusch, 2003).

It has been well established that both food insecurity and malnutrion increase susceptibility and vulnerability to HIV infection especially amongst women (Kadiyala and Gillespie, 2003;

Krishnan et al., 2008). Women in resource-constrained settings are usually the caregivers of large extended families and therefore often bear the brunt of food shortages and become especially prone to nutritional deficiencies (Black et al, 2008).

Melchior et al (1993) compared resting energy expenditure (REE) in a group of malnourished HIV infected individuals to a group of controls, and found that REE was higher in the individuals with secondary infections as compared to stable HIV infected individuals and that the stable HIV infected individuals in turn also had a higher REE when compared to the normal controls. This was reiterated by Grinspoon et al (1998) who found that the resting energy expenditure in HIV-infected women was higher than their HIV-uninfected control group. These findings led to the conclusion that HIV positive individuals had increased energy requirements.

Wasting has been well documented as a marker of progression to AIDS. Suttman et al (1995) observed that it was a stong predictor of survival independent of the CD4 count. This was reiterated in a longitudinal follow-up of HIV positive individuals by Melchior et al (1999). They found not unsurprisingly that after controlling for other factors, only CD4 counts, lean body mass/height squared and CRP were independent predictors of survival.

Micronutrient deficiencies are common in the presence of HIV infection, due to a variety of causes such as anorexia due to the disease itself; inadequate dietary intake due to reduced availability; increased nutrient requirements due to increased metabolism; and decreased absorption due to opportunistic infections (Semba and Tang, 1999; Gillespie and Kadiyala, 2005; Koethe and Heimburger, 2010).

HIV has therefore both a biological and a non biological effect on the nutritional status of individuals; the biological effect being the direct effect of HIV on nutrition due to the increased energy requirements as well as reduced absorption/intake of nutrients due to the increased prevalence of opportunistic infections; and the non-biological effect being the effect of HIV infection at the family and community level, as it affects mostly young men and women who are often the sole breadwinners/ caregivers resulting in the breakdown of the family structure as well as loss of income and skills (Panagides et al., 2007).

1.3 Impact of lactation on health and nutritional status of women

Nutrient needs during lactation are considerably higher than those during pregnancy and are proportional to the intensity and duration of breastfeeding. Ideally, some of the nutrients stored during pregnancy are mobilised during lactation, including energy stored as fat. Butte and King (2005) in a review of different methods and measures of energy requirements and changes in metabolism; summarized that requirements of lactating mothers who are exclusively breastfeeding are increased by approximately 626 kcal/day, based on a mean milk production of 749g/day. In normal healthy mothers, this can be mobilized from their tissues at the rate of172 kcal/day. If a mother has a low BMI during pregnancy or has a disease with additional metabolic demands like TB or HIV, energy will need to be consumed daily to compensate for deficit of additional stores. As with pregnancy, several metabolic adaptations meet the metabolic needs of lactation. These include increased appetite and food intake, mobilization of tissue stores, increased metabolic efficiency and reduced energy expenditure.

Early research from Nduati et al (2001) in Nairobi, Kenya, provided some disconcerting data that HIV infected breastfeeding mothers had higher 24 month mortality than their formula feeding counterparts. The researchers attributed the cause of their findings to two factors,

firstly they proposed that combined metabolic burdens of HIV-1 infection and breastfeeding in a population that has inadequate nutritional intake could lead to substantial nutritional impairment and secondly they suggested that lactation might affect HIV-1 replication. In an accompanying commentary of the study by Newell (2001) it was pointed out that the data needed to be interpreted with caution because of limitations in the study. In spite of the limitations of the study it was obviously important that such a finding was further investigated to test its validity.

A study undertaken in Tanzania (Sedgh et al, 2004) showed in multivariate analyses that neither breastfeeding status nor the duration of exclusive or partial breastfeeding was associated with maternal HIV-1 disease progression amongst the mothers (represented by death or development of a low CD4 cell count, anaemia or excessive weight loss). These associations remained insignificant when women with relatively low and high CD4 cell counts were analyzed separately. A meta-analysis involving 9 large studies (BHITS, 2005) also refuted the Nairobi findings and suggested that breastfeeding does not pose any mortality risk to the HIV infected mother. Since then, two other studies with longitudinal follow-up to two years found no greater maternal mortality associated with breastfeeding (Kuhn et al, 2005; Coutsoudis et al, 2010).

Although there appears to be strong evidence that lactation has no adverse impact on mortality in HIV infected women there is insufficient evidence on the impact of lactation on the nutritional status of these women. Papathakis et al (2006) showed that the HIV positive lactating mothers lost more weight and subcutaneous fat than their HIV negative equivalent, however there was no difference between the fat free mass in both groups.

Because there is some concern and conflicting evidence that lactation is a metabolic drain on HIV infected women from impoverished backgrounds, we investigated the impact of lactation on body composition of HIV infected lactating mothers compared to non-lactating mothers in a semi-urban community.

Considerable evidence exists to show that in HIV infected individuals it is not sufficient to simply monitor weight and height changes when testing the impact of interventions/practices and instead changes in body composition provide far more information (Kotler et al, 1989).

1.4 Impact of nutritional interventions on general health outcomes of HIV infected lactating women

Macallan (1999) in a review stated that the complex interaction between HIV, immune status and nutrition, could be to our advantage as it would help design strategies that could improve the health and perhaps even slow disease progression in the infected individuals. Fawzi et al (1998) found that supplementation of HIV positive pregnant women with multivitamins (excluding vitamin A) at multiple RDA (required dietary allowance) when compared to placebo reduced adverse pregnancy outcomes.

Piwoz and Preble (2000) have shown in their review that there are increased requirements for both macronutrient and micronutrients for people living with HIV/AIDS. They estimate a 50% increase in protein intake and a 15% in the energy intake. A Cochrane review on the different trials of micronutrient supplementation in children and adults with HIV concluded that there was no evidence at present to recommend any one micronutrient intervention to reduce morbidity and mortality associated with HIV in adults; however there was some evidence that vitamin A supplementation in children was beneficial (Irlam et al, 2005).

Kongnyuy et al (2009) in a systematic review of all published literature concluded there was no evidence to support vitamin A supplementation to HIV infected mothers despite an improvement in birth weight. Since then the Tanzanian group repeated their earlier study using single RDAs of multivitamin and have reported that multivitamins (excluding vitamin A) at even the single RDA significantly reduced the risk of adverse pregnancy outcomes (Kawai et al, 2010).

1.5 Impact of nutritional intervention on mother's mental health

It is very useful to be able to establish what type of additive psychological effects the nutrition supplementation may have on the mother and her baby. Since food security is one of the main issues which affect the day to day living in the impoverished household, it is proposed that alleviation of such a stressor through nutritive intervention may impact positively on the mother's emotional disposition. The mother's mental health status can be monitored using the WHO self reporting questionnaire (SRQ20), which has been validated by the researchers at the University of KwaZulu-Natal in our study population (Zulu) (Bhagwanjee et al, 1998; Harpham, 2003). The mother's mental health status may positively affect the level of care she gives to her baby, thereby improving the baby's growth and development (Moses-Kolko and Roth, 2004; Boyd et al, 2006). Maternal survival and wellbeing has been shown to be essential for infant survival (McDermott, 1996). This study will therefore provide important information on the availability of affordable and practical interventions to improve overall maternal health and subsequent child health and nutrition.

1.6 Impact of nutritional intervention on body composition

It has been shown that nutritional supplementation during pregnancy especially in the

under-nourished population improves pregnancy outcomes and birth weight. Cisse et al (2002) showed that nutritional supplementation during pregnancy improved fat free mass in their population, however her population was HIV uninfected. In the Gambian breastfeeding mothers, Prentice et al (1980) observed that maternal supplementation increased subcutaneous fat stores and maternal body weight.

There was no published literature available on the effects of nutritional supplementation on body composition changes during pregnancy or lactation in HIV infected women.

1.7 Impact of nutritional intervention on the breastmilk composition of HIV infected lactating women

Jelliffe and Jelliffe (1978) in a review of the available literature on breastmilk volume and composition stated that although well nourished mothers produced enough milk to exclusively breastfeed their infant for six months without any supplementation; undernourished or starving women need additional supplementation during pregnancy and lactation to improve the quality and volume of their breastmilk. Prentice et al (1980) in their study of the effects of a nutritional supplement to Gambian nursing mothers observed that the supplement did not increase breastmilk output or fat content.

The effect of inadequate intake of various nutrients on the success of lactation, and infant health remains largely unknown. A review by Allen (1994) showed that maternal micronutrient deficiencies are likely to affect breast-milk composition. In general, breast-milk content of water-soluble vitamins is most affected by inadequate maternal intake whereas it is less affected by maternal intake of fat-soluble vitamins or minerals.

Studies of high dose post-partum supplementation with vitamin A to breastfeeding mothers have shown that it significantly increased the breast milk retinol levels (Bahl et al, 2002). Brown et al (2009) examined the literature on levels of zinc in breastmilk over time and compared it to the requirements of an infant. They concluded that breastmilk supplies a sufficient amount of zinc for several months and even after six months, although the zinc levels in the milk may have declined, it continues to supply at least half the child's requirements in normal populations. Most studies of zinc supplementation to lactating mothers have however shown that it did not make any impact on the zinc levels in the breastmilk (Chierici, 1999).

In a Senegalese study on nutritional supplementation during pregnancy amongst undernourished women, the investigators observed a significant increase in the levels of lactose, protein and zinc in the breastmilk of the supplemented mothers (Cisse et al, 2002).

1.8 Impact of nutritional intervention to lactating mothers on infant growth and health

In a review of interventions to improve growth and development of children, WHO (1999) outlined reasons why and how breastfeeding has a beneficial effect on the growth and development of infants due to increased stimulation and bonding between the mother and child; there was however no supplementation involved.

Christian (2010) in a review of published literature and trials on multi micronutrient supplementation of pregnant mothers states that although the trials showed a 11 % reduction in low birth weight there was no effect on survival; therefore there is no evidence at present to advise multiple micronutrient supplementation to mothers instead of the current iron-folic acid combination.

Helland et al (2008) in a trial of supplementation with very long fatty acids on pregnant and lactating women showed although there was no effect on their children's global IQs (Intelligence quotients); there seemed to be some effect on cognitive function at 7 years of age. In a placebo controlled trial with factorial design on HIV positive lactating women, Fawzi et al (2003) showed that the infants born to mothers using multivitamin supplements had a significantly lower risk of diarrhoea than those in the no- vitamin arm. Vitamin A had no effect on the incidence of diarrhoea however it did reduce the risk of pneumonia significantly. They also found that maternal vitamin A supplementation increased the risk of vertical transmission. They have subsequently reported that vitamin A supplementation during lactation seemed to be associated increased viral shedding in breastmilk (Villamor et al, 2010); and that both multivitamin and vitamin A supplementation is associated with subclinical mastitis (Arsenault et al, 2010).

1.9 Infant Feeding Practices

At the time of conducting this study the South African HIV and Infant Feeding guidelines were based on the 2007 WHO/UNICEF/UNAIDS guidelines which we therefore followed. Women who chose to breastfeed in both groups viz. with or without nutritional supplements, were counseled and supported to practice exclusive breastfeeding for 6 months, and thereafter mothers with infants who tested HIV negative were counseled on either discontinuing breastfeeding at 6 months, or heat treating expressed breast milk (HTEBM) if they do not satisfy all the AFASS criteria for replacement feeding (Coutsoudis, 2005; Israel-Ballard, 2007; Mbuya et al, 2010).

1.10 Techniques for measuring body composition

There are various techniques available to assess and monitor body composition. Until recently most body composition studies in developing countries have been limited to the use of anthropometric techniques and invalidated bioimpedance analyses (BIA).

BIA is a method by which electrodes are attached to the wrist and ankle and the difference in the voltage between the electrodes is measured; this measurement is converted into a total body water and lean body mass reading using an empiric equation inbuilt into the machine by the manufacturer. These equations have been derived in western populations against a reference method. Prediction formulas for body composition tend to be population-specific (Ward et al, 2000; Deurenberg et al, 2002). Previously obtained BIA results based on white subjects, might be inappropriate in black populations and need to be retested. Dioum et al (2005) showed that BIA prediction equations for total body water differ between African Americans and Whites, but the reasons for this remain unclear.

Simple, valid methods that are reproducible are required to assess body composition in HIV-infected African women. Amongst the different reference methods available for assessment of body composition, dilution techniques using stable isotopes are now considered to be the more practical and acceptable in terms of minimal discomfort to the subjects (Jebb and Elia, 1993; Jennings et al, 1999). Deuterium Oxide and ^{18}Oxygen are two stable isotopes that have both been safely used, however as Deuterium Oxide is relatively much cheaper it is used more frequently in body composition studies. Papathakis et al (2005) showed that body composition estimates obtained by bioelectrical impedance assay (BIA) together with anthropometry were comparable to the deuterium dilution method and that body mass index and mid upper arm circumference are useful in predicting fat mass in HIV-infected and

uninfected women.

However BIA was not useful in determining lean mass in HIV-infected women. Therefore, in order to elucidate more clearly the impact of nutritional interventions/practices on the body composition it would be better to use the gold standard viz. deuterium dilution methods and standardize the BIA measurements. Lean mass or fat free mass is of particular interest in the background of HIV-infection and studies of HIV infected individuals have suggested that mortality is correlated with loss of lean tissue rather than overall weight loss (Kotler et al, 1989; Melchior et al, 1999).

1.11 Motivation for study

There are no published studies on the impact of nutritional/food supplements on lactating HIV infected women and, as discussed earlier, HIV and lactation independently exercise a toll on the lactating women in terms of energy costs. There is therefore a need to test the effect of circumventing this energy loss by providing a well- balanced nutritional supplement.
As all women in the study received a multivitamin supplement (as part of the national PMTCT programme recommendations) we were not in a position to test the impact of micronutrients on health and nutritional status but instead we tested the impact of providing a food supplement in conjunction with the multivitamin on the nutritional status of the breastfeeding mothers.

We decided to test a food supplement in the form of a peanut/soya milk based spread enriched with micronutrients. The paste is ready to eat and can be eaten on its own, spread on bread or added to porridge. It has a shelf life of 8 months and once opened it has a shelf life of up to 3 weeks at room temperature. The manufacturers supplied the paste to us free of charge. The

daily serving of the paste was 50g which supplied 280kcal energy and 8g protein (appendix 1). It has been enriched with multivitamins as well iron, zinc, selenium, copper, phosphorous, magnesium, iodine etc and supplies between 23 to 100% of the RDA (recommended dietary allowance) for most of the micronutrients e.g. vitamin A(75%); folic acid (75%); B12 (60%); iron (100%) and zinc (33%).

The paste was being used by the national department of health as a therapeutic supplement in malnutrition and HIV associated wasting; we therefore assumed that it was acceptable in taste and texture to our community. Additionally there were plans by the provincial department of health to provide this paste in food parcels for HIV infected lactating women however during the study period, our clinic programme did not receive any of these food parcels.

In order to be able to test the effect of nutritional support on the health of the mothers we randomly allocated breastfeeding women to either receive the paste or to receive non-nutritive consumables such as tea and soap products of equal monetary value.

We planned to assess the impact of the food supplement on the mothers' nutritional status (anthropometrics and body composition and micronutrient levels in plasma and breastmilk); disease progression (changes in CD4 counts and WHO disease stage) and on mental health (SRQ20: self reporting questionnaire; see appendix 4, page 161). In addition we used the opportunity to also compare all these parameters (except breastmilk macro- and micronutrients) in the breastfeeding women vs. another group of HIV infected women who chose not to breastfeed. We also planned to do cervical smears at six months in all the mothers to assess for Human Papilloma virus (HPV) infection which is considered to be a prognostic indicator in sexually active HIV positive women (Kawonga and Fonn, 2008).

Infants born to the mothers in these 3 study groups (non-breastfeeding; breastfeeding without nutrient supplement; and breastfeeding with nutrient supplement) were assessed in terms of nutritional status and morbidity monitoring at 2, 6, 10, 14, 24 weeks and thereafter at 3 monthly intervals until the infants were 9 months of age. In addition development being an essential indicator of infant well-being was assessed over the study period using a modified Denver developmental screening test and a truncated Denver developmental score. Within these 3 groups of infants we also examined these parameters in sub-groups of infants who were HIV infected and those who were not.

1.12 Overall objective

To assess the effect of breastfeeding and maternal nutritional supplementation on maternal and infant health in the presence of HIV infection and exposure respectively.

*1.12.1 Primary o*bjectives

1. To study the effect of breastfeeding on the nutritional status, disease progression and psychological indices of HIV positive mothers

2. To study the effect of nutritional supplementation to lactating HIV positive mothers on the nutritional status, disease progression and psychological indices of the mothers

3. To study the effect of breastfeeding on the growth; development; and incidence of opportunistic infections in the HIV exposed negative infants

4. To study the effect of nutritional supplementation to lactating HIV positive mothers on the growth, development, and incidence of opportunistic infections in the negative infants

1.12.2 Secondary objectives

1. To study the timing of HIV-1 infection; and disease progression in the HIV infected infants

2. To study the effect of nutritional supplementation in breastfeeding mothers on the quality of breastmilk i.e. the protein and micronutrient content of the breastmilk.

3. To formulate a predictive population specific equation for calculation of Lean body mass through bioelectrical impedance measurements standardized against the gold standard (saturation of Deuterated water).

1.13 Hypotheses tested

- Breastfeeding will not negatively impact on mothers' lean body mass; nutritional status; disease progression and psychological indices.
- Nutritional supplementation provided to HIV infected lactating women will improve their lean body mass; nutritional status and health.
- Exclusive breastfeeding vs. formula feeding will reduce diarrhoeal morbidity; improve growth and developmental indices in the HIV exposed negative infants and would improve outcome in HIV infected infants.
- Nutritional supplementation provided to HIV infected lactating women will improve the quality of breastmilk and may therefore have an impact on the growth and development of their infants.

Chapter 2

Methods

2.1 Study population

The study sample was obtained from pregnant women attending the antenatal care services at Cato Manor clinic also known as Umkhumbane Community Health centre. This centre serves the community of Cato Manor which covers an area of 1800 ha and has an approximate catchment population of 123, 600 and comprising mainly people of Zulu ethnicity. Pregnant women who presented for delivery after hours when the maternity unit of the Cato Manor Clinic was not in operation were referred to King Edward Hospital which is a few kilometers away. Mothers then returned to Cato Manor Clinic for their follow-up visits.

2.2 Study design

The study had 2 components; a prospective study to examine the impact of feeding mode on various indices and within it a nested randomized clinical trial to study the impact of nutritional supplementation on the breastfeeding mothers.

In the nested randomized trial, the breastfeeding mothers were randomized to receive supplementation or to receive non-nutritive household supplies. The nutritional supplementation was a peanut/soya milk food paste enriched with micronutrients packaged in plastic tubs (appendix 1). The product label of the food paste was covered to prevent inadvertent promotion of the investigated product. Non-nutritive supplies consisted of tea; shampoo and conditioner of a similar monetary value. The supplement and the non-nutritive supplies were packed in identical brown bags in an attempt to mask the allocation of group from the principal investigator as well as from each other (participants). The bags were issued

monthly from two weeks to six months by a dedicated study counselor who maintained a study register with the randomisation allocation and signatures from each participant on receipt of the bag at each visit to ensure compliance. The counselor also did an adherence questionnaire with each participant to check if they were using the supplement and the reasons for non-compliance if any (appendix 4, page 168). Care was taken to ensure that the principal investigator was kept blinded to the randomisation groups as study numbers were used for data entry and for the study samples analysed.

The group receiving household supplies acted as the control group for the group receiving supplementation. In order to prevent dilution of the effect of the intervention; we allocated separate clinic visit days for the three groups.

In summary we investigated health and nutritional changes in mothers and their infants over a nine month period in the following three groups:

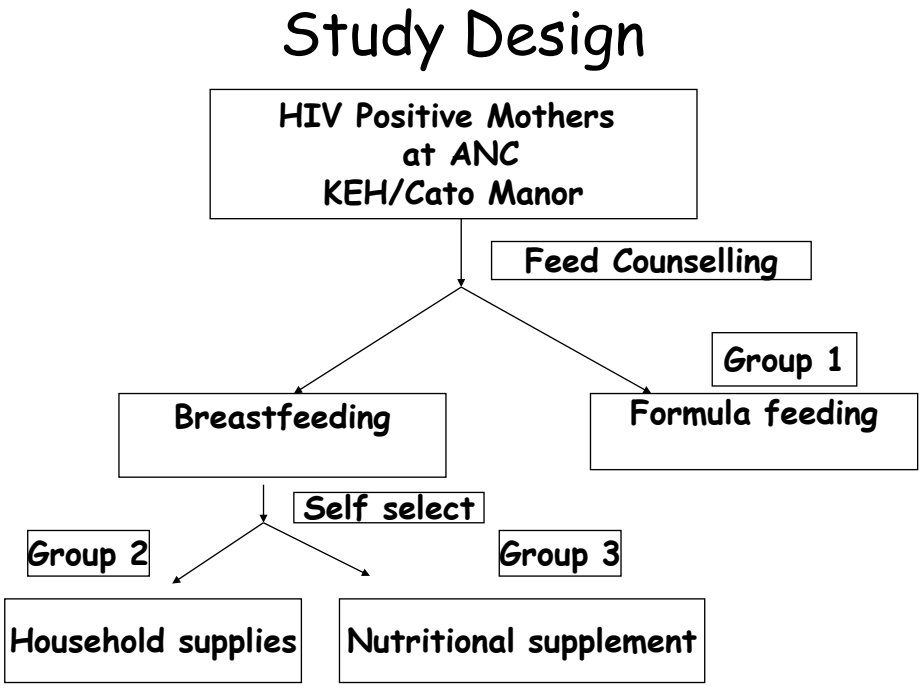
Group 1: HIV-infected formula feeding (non-breastfeeding) women

Group 2: HIV-infected breastfeeding women not receiving nutritional supplement

Group 3: HIV-infected breastfeeding women receiving nutritional supplement for 6 months.

The study design is summarized in figure 1 below:

Figure 1: Study design



2.3 Randomisation

In order to ensure mothers had confidence that the randomization was transparent, we printed a complete batch of Group 2 and Group 3 tag cards and placed them in a closed box, this was shuffled at each mother's enrolment and the mother was asked to pick out a card resulting in the mother randomizing herself to a group.

2.4 Sample size calculations

To calculate the sample size, it was hypothesized that a difference of 4kg in lean body mass over a 6 month period between the 2 breastfeeding groups would be clinically significant. We therefore calculated a sample size that would be needed to show a statistically significant difference between the groups based on this difference.

The null hypothesis (H0) we proposed was that the difference between the mean lean body mass in the two groups' viz. group 2 (breastfeeding control) and group 3 (breastfeeding supplemented) would be less than or equal to 4 kg. The alternate hypothesis (H1) was that the difference would be more than 4 kg.

Calculations that were used to derive the sample size:

Power Analysis of the Difference of Two Means

Numeric Results (H0: D <= -|E|; H1: D > -|E|)

Test Statistic: T-Test

Power	N3/N2	Equivalence Margin (E)	Actual Difference (D)	Significance Level (Alpha)	Beta	Standard Deviation3 (SD3)	Standard Deviation2 (SD2)
0.90545	40/40	-4.00	0.00	0.02500	0.09455	6.70	3.60

Definitions used in the calculations:

Group 3 is the treatment group. Group 2 is the reference or standard group.

Power is the probability of rejecting a false null hypothesis. Power should be close to one.

N3 is the number of subjects in the first (treatment) group.

N2 is the number of subjects in the second (reference) group.

|E| is the magnitude of the margin of equivalence. It is the largest difference that is not of practical significance.

D is the mean difference at which the power is computed. D = Mean3 - Mean2.

Alpha is the probability of a false-positive result.

Beta is the probability of a false-negative result.

SD3 and SD2 are the standard deviations of groups 3 and 2, respectively.

Calculations result:

As seen above it was determined that a sample size of 40 in each group would achieve 91% power to detect this difference using a one-sided, two-sample t-test. The true difference between the means is assumed to be 0.00. The significance level (alpha) of the test is 0.02500. The data are drawn from populations with standard deviations of 6.70 and 3.60.

Allowing for a 30% loss to follow-up due to high mobility of the population post delivery, we estimated a sample size of 52 in each group. Then adding another additional 30% (of the original 40) for loss of data due to missed visits for body composition analyses or laboratory tests; we estimated a sample size of 64 in each group.

2.5 Ethical approval

Ethical approval for the study was obtained from the Bioethics Committee of the University of KwaZulu-Natal (H081/05; appendix 2). Permission was also obtained from the King Edward VIII Hospital administration, the Ethekwini Municipality and Cato Manor clinic for conducting the study.

2.6 Exclusion criteria

- Women who had advanced disease (CD4 < 200 or WHO stage 3 and 4 disease) were not eligible for participation and were referred to the ARV treatment programme on site.
- Women who did not intend to stay within a 30 km radius of the clinic and would not be able to attend follow up visits
- Any mother who gave birth to a baby with any congenital abnormality or with any complication during birth requiring specialized management
- Gestation less than 36 weeks

2.7 Study recruitment

All mothers attending the antenatal clinic at Cato Manor clinic are pre-counselled and tested for HIV-1 infection after an informed consent is obtained (appendix 3). Those testing positive are referred to the on site MTCT Plus programme, an internationally funded programme that provides PMTCT services as well as comprehensive care and treatment for HIV infected mothers and their families.

After mothers received post-test counselling from the MTCT Plus staff, they were referred to study counsellors who discussed the study with them. They were then seen at their next routine antenatal clinic visit (ANC) and asked if they had any queries about the study; their CD4 counts were checked as well at this point to assess for study eligibility criteria; and at this point all women with low CD4 counts or WHO stage 3 or 4 were directed to the MTCT Plus programme for further management.

The women who were considered eligible for our study and who remained interested were then seen at each routine ANC visit for infant feeding counselling and for any new queries and continued interest in the study. Pre-enrolment was done before delivery after an informed consent was obtained as well as collection of baseline socio-demographic data, discussion of infant feeding choice and obtaining contact details (appendix 3-4). All the mothers were counselled on the feeding options and risks thereof were explained. They were then asked to return 2 weeks post delivery with their infants. They were officially enrolled into the study post delivery when the 1st study measurements were done. Due to the high mobility of the community, it was decided at the outset, that only those mothers who return for at least 2 or more study visits would be included in our analysis.

2.8 PMTCT prophylaxis

All mothers attending the clinic received a single dose Nevirapine (sd NVP) for PMTCT prophylaxis as per national guidelines in 2006 from the antenatal clinic. The mothers who presented to ANC clinic early received prophylaxis with Combivir ® from 36 weeks of gestation continued to one week post delivery (from MTCT Plus programme) in addition to the sd NVP. All babies received sd NVP within 72 hours of delivery.

2.9 Study visits and procedures

A questionnaire was administered to all mothers at baseline to establish fulfilment of AFASS criteria, socio-demographic indicators, parity, assessment of food security, and history of any sexually transmitted disease (STI) during the current pregnancy as well as history of TB in last two years. WHO based definitions was used to establish AFASS and food security status. STI is associated with vertical transmission and TB is an important marker of immune status as all are exposed to TB in a developing country, however it presents as a disease in those whose immune status is compromised, therefore information was obtained on the above.

Post delivery, a delivery history as well as a clinical examination and anthropometric measurements were performed on the mother and child, besides, this body composition studies were performed on the mother.

2.9.1 Babies

All babies were seen at 2 and 6 weeks post-delivery and monthly thereafter till 6 months of age and the final study visit was conducted at 9 months. At each visit, a clinical examination, developmental assessment and anthropometric measurements were done. A dry blood spot specimen was obtained for HIV DNA PCR at 6 weeks as per national protocol. All DNA PCR

positive babies were retested at the next visit 10 weeks and started on antiretroviral therapy as per national guidelines. HIV-1 exposed PCR negative breast fed babies irrespective of the randomisation were tested again at 9 months to rule out breast milk transmission. The HIV-1 exposed and infected babies were assessed for any signs of disease progression and opportunistic infections, WHO disease staging at each visit and CD4 counts 6 monthly. A full feeding history was also taken at each visit.

Multivitamin supplementation and Cotrimoxazole prophylaxis: All the infants received multivitamin syrup provided by the clinic as per national guidelines from six weeks of age. They also received cotrimoxazole for *Pneumocystis jiroveci* pneumonia (PCP) prophylaxis from six weeks which was stopped when the infant was confirmed HIV DNA PCR negative.

2.9.2 Mothers

All mothers were seen at two weeks, six weeks and monthly thereafter till six months, with a final visit at nine months. At each visit a clinical and nutritional assessment was conducted to identify signs of disease progression and opportunistic infections. WHO disease stage and a Karnofsky score was determined at each visit. Laboratory assessments were done at two weeks and six months. The assessments included haematological indices, total protein, albumin, Zinc, Folate and Vit B12 (2.14). Body composition measurements using Deuterium enrichment and a multi-frequency bioelectrical impedance analyser were done at the two week and at three, six, and nine month visits (2.11-2.12). Cervical smears and CD4 counts were done at six months. A 24 hour dietary recall was done at three, six and nine months to assess for similarity of dietary intake in the groups to rule out any confounding for the outcome of supplementation (2.13). Quality of life was assessed using Karnofsky scoring and

mental status using the SRQ 20; both of which had been validated by WHO for use in the HIV population.

Mothers whose disease progressed were assessed for ART eligibility at each visit and started on treatment by the externally funded HIV care and treatment programme operating at the clinic if indicated as per national guidelines. The final study visit was conducted at nine months; at this stage they were given their CD4 counts and referred to continue with the care and treatment program on site.

Multivitamin supplementation: All the mothers received a multivitamin supplement provided by the clinic as per national guidelines (appendix 5).

2.10 Anthropometry methods

Anthropometric measurements performed for the mothers and infants included weight; height; length; mid-upper arm circumference (MUAC); and triceps skinfold (TSF) thickness. In addition, head circumference measurements were done on the infants. To reduce inter-observer variability and enhance reliability, all anthropometric measurements were performed by either the principal investigator or the dietitian. Anthropometric measurements were performed at the following visits: two; six; ten; and fourteen weeks; four; five; six; and nine months. Standardised techniques and the same equipment were used for all measurements. All anthropometric measurements were performed according to the ISAK (International Society for the Advancement of Kinanthropometry, 2001) methods. The weight, height, MUAC and TSF measurements were taken in duplicate and the mean of the two measurements was used. To enhance precision, if the two measurements differed by more

than 0.1 kg for weight for adults or by more than 0.05 kg for infants; or by more than 0.5 cm for height, length, MUAC and TSF measurements the measurements were repeated.

2.10.1 Adults

Weight was measured using an electronic scale (SECA 882) and recorded to the nearest 0.1 kg. All subjects were required to remove their shoes and all exterior clothing; some light clothing was allowed (Lee and Nieman, 2003). Height was measured using a stadiometer (SECA 225) and recorded to the nearest 0.1cm. Subjects were measured without shoes and standing in an upright position. The head was positioned in the Frankfort horizontal plane and the subject was asked to relax their shoulders and stand with their arms at their sides (Lee and Nieman, 2003).

The MUAC was measured using a flexible, inelastic tape measure (SECA) and was taken on the right side for all subjects. The mid-point between the top of the acromion process of the scapula and the olecranon process of the ulna was located and a mark was made using a marking pen. The MUAC was then measured to the nearest 0.1cm with the arm hanging freely at the subject's side (WHO, 1995). Lange Calipers were used to measure the TSF, which was measured to the nearest mm. All measurements were made on the right side of the body (Norton and Olds, 2001). The Body Mass Index (BMI) was calculated by dividing the weight of the subject by their height squared (kg/m^2).

2.10.2 Infants

Infants were weighed using an electronic infant scale (Misaki, Japan); weight was recorded to the nearest 0.05 kg. All clothing was removed and the infants were weighed wearing only a dry disposable napkin. Length was measured using a length board with a movable headboard

that was specially built for the purposes of the study. Two observers performed the measurement, one held the feet flat against the fixed board and the other held the head still and read the measurement. Length was recorded to the nearest 0.1cm.

Head circumference was measured using the SECA head circumference tape. The infant was held on the mattress in a sitting position by the mother; with the head held in the Frankfort plane, the measurement was made along the maximum circumference using the supra-orbital ridges in the front and the occipital protuberance at the back of the head as surface markers. The measurement was made to the nearest 0.01 cm.

The MUAC was measured using a flexible, inelastic tape measure (SECA) and was taken on the right side for all subjects. The mid-point between the top of the acromion process of the scapula and the olecranon process of the ulna was located and marked using a marking pen. The MUAC was then measured to the nearest 0.1cm with the help of an assistant to hold the infant as still as possible (WHO, 1995). TSF measurements were performed using Lange Calipers to the nearest mm.

All measurements were taken in duplicate and the mean value calculated and entered into the database. The data was analysed using WHO 2007 growth standards using the WHO 'igrowup' tool (WHO 2009a).

2.11 BIA methods

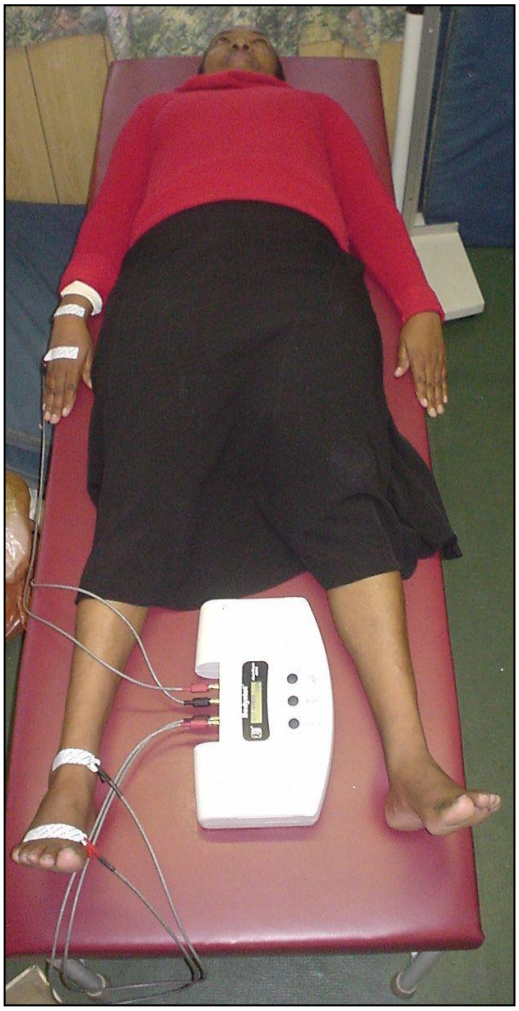
BIA was performed for all the mothers at the 2 and 14 week visits and the 6 and 9 month visits. A quad-frequency analyser (Bodystat® QuadScan 4000 Hydration/ Body Composition Monitoring Unit, Isle of Man, British Isles) was used. To enhance reliability, all the

assessments were performed by either the principal investigator or the dietitian and the analyser was calibrated before each analysis using the calibrator supplied by the manufacturer.

Measurements were performed according to the manufacturer's instructions using standardised procedures and electrode placement. The skin surfaces on which the electrodes were placed were cleaned with an alcohol swab to ensure good adhesion and the disposable electrodes were then attached to the wrist, hand, ankle and foot on the right side of the body (see figure 2). Measurements were done with the subject lying supine, with limbs slightly abducted and analysis was performed after the subject had been lying in the supine position for five minutes (Bodystat® QuadScan 4000 User's Guide). All measurements were done in duplicate and a mean value was calculated. Measurements were repeated if there was a difference of more than 5 units in the impedance measurements.

Subjects came in the morning fasting for an hour for the Deuterium enrichment samples and were kept fasting for the next four hours for the remainder of the samples. The BIA measurements were conducted at the end of this time period in keeping with the requirements that the subjects were fasting for a minimum of four hours prior to the measurements (Wanke et al, 2002). All subjects were measured around the same time at each visit, to rule out any diurnal variations. A standardised form was used to record the BIA measurements (appendix 4).

Figure 2: Positioning of electrodes on subject



2.12 Deuterium Enrichment Methodology to determine Body Composition

Deuterium oxide ($^2H_20$) is water in which 99.8 or 99.9% of the hydrogen atoms are in the form of deuterium; it is also called D_2O. The mass of hydrogen is 1 and that of deuterium is 2. No side-effects have been noted with the amount used for dilution techniques.

Principle of method: The principle of isotope dilution is that when a known quantity of a labelled compound is added to a biological system and mixes with that pool; after a few hours the enrichment reaches a plateau and the dilution of the labelled compound by the endogenous unlabelled compound will give us the size of the pool. The pool, also called volume of distribution (V_D), is larger than the total body water (TBW) due to non-aqueous exchange. Non-aqueous exchange is a process by which the isotopes in body water exchange with hydrogen atoms in other body components- protein, fat etc. V_D has been estimated to be 1.041 times the TBW. Therefore TBW (kg) is obtained by dividing V_D by 1.041. TBW contains both intracellular and extracellular fluid. It usually represents 50-60% of body weight in lean adults and less than 40% in obese.

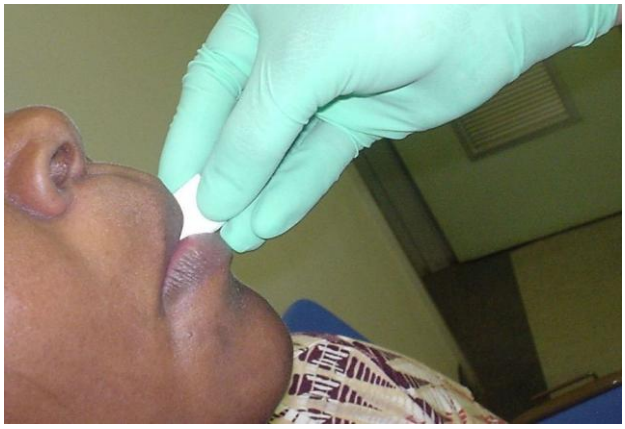
The natural abundance of deuterium is 0.015%. Therefore it is essential to do a baseline saliva sample to calculate the background. Enrichment is the concentration of deuterium above the baseline sample. The FTIR subtracts the background from the reading obtained from the post-dose sample.

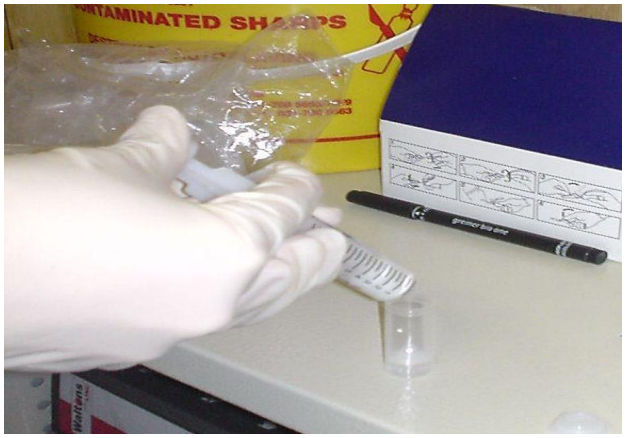
The fat free mass or lean body mass (LBM) is approximately 73.2% of the TBW. Therefore we can calculate the LBM from the TBW. The fat mass is the difference between the body weight and the LBM.

2.12.1 Collection of saliva samples and administering deuterium

A standardized operating procedure based on International Atomic Energy Agency Guidelines (Human health series document: "Assessment of body composition and total energy expenditure in humans by stable isotope techniques") was developed and followed for collection of the samples (appendix 6). Saliva samples from mothers were collected to measure Deuterium enrichment at 2 weeks; and 3; 6; and 9 months. A baseline sample was collected at each visit followed by a dose of 20g Deuterium. Subsequent saliva samples were collected at 3 hours and 4 hours after ingestion of the deuterium dose. See figure 3 for photographs of the saliva sampling and deuterium dosing. All samples were stored in a -20°C freezer. Samples were then batched and analysed using an FTIR (Fourier transform Infrared spectroscopy) instrument. The instrument was housed in the Department of Chemistry, University of KwaZulu-Natal.

Figure 3: Photographs of saliva sampling and deuterium dosing





2.12.2 FTIR methodology

The underlying principle of Infrared spectroscopy is that when an infrared radiation is passed through a sample while a portion is absorbed by the sample the remainder is transmitted. Each substance has its own unique spectrum which represents its molecular absorption and transmission. This principle is used to determine composition of various specimens and other analyses.

Principles of the FTIR: Constituents of the FTIR include a source of infrared radiation; a beam splitter; two mirrors (one fixed and one moving); and a detector. The interferometer comprises the beam splitter and the two mirrors. The moving mirror is mounted on an assembly and moves back and forth at a constant velocity. The beam splitter in effect splits the radiation sending half to the fixed mirror and the other half to the moving mirror. The two mirrors then reflect the radiation back to the beam splitter where they recombine. This recombination forms an interference pattern the sum of which is called an interferogram. This interferogram is then passed through the sample and focussed on the detector. As the interferogram is comprised of the sum of cosine waves obtained at different wavelengths of the radiation; some of the frequencies are absorbed by the sample more than the others. This therefore modifies the interferogram differently for different specimens. This is analysed through a mathematical method available as a programme. The Shimadzu IRSOLUTION software was used. The software comprises of two files “isotope exe” and “vbrun300.dll” (appendix 7)

Figure 4: Shimadzu FTIR-8400S

2.12.3 Development of a population specific prediction equation for use in BIA determinations of Lean Body Mass

The BIA has inbuilt equations which calculate the total body water, however these equations are not population specific and may need adjustment for our population. Hence, using the total body water obtained through deuterium enrichment as gold standard, we planned to develop regression population specific equations for use in the field.

A spreadsheet containing all anthropometric measurements, values of TBW obtained from the BIA and FTIR as well as the impedance values obtained from the BIA at 5, 50, 100 and 200 kHz was imported into SPSS. A univariate analysis was carried out for the continuous variables to check for normality of distribution. Following this a bivariate analysis was carried out to check for any associations between TBW obtained through FTIR and all the other measurements. The dataset was then randomly partitioned into two portions- one was labeled the development sample and the other the validation sample. Using stepwise linear regression on the development sample using the variables with the strongest Pearson's correlation coefficient, a model was obtained. The model was then applied to the validation sample and an adjusted predicted value for TBW was obtained. A paired t test was done between TBW obtained from the FTIR and the adjusted predicted value obtained from the model to estimate the bias, deviation of the bias and 95% confidence intervals. Values obtained from the two assays were compared using a Bland-Altman Plot, by plotting the difference between the values against the mean of the values. The same methodology was used to form an equation using the TBW obtained from the BIA and from the anthropometric measurements alone.

2.13 Dietary Assessment

The dietary assessment was carried out at three time points for each subject/ mother. Dietary intake was assessed by means of multiple 24-hour recalls carried out at the 14 week, six month and nine month visits. The dietary intake assessment was carried out by the dietitian. A trained Zulu-speaking counselor assisted with translation when necessary.

We used the Dietary Assessment and Education Kit (DAEK) and FoodFinder 3 to determine the dietary intake. The FoodFinder3 for Windows® is a software programme developed by the Nutritional Intervention Research Unit (NIRU) and Biomedical Informatics Research Division (BIRD) of the South African Medical Research Council (MRC) in collaboration with WAMTechnology CC. It is based on MS Access format thus allowing capture of longitudinal data.

The FoodFinder3 included the latest South African food composition database with its latest updates (1998-2000) and the 1991 food composition tables. The energy calculations in the programme are based on the energy conversion factors for protein, carbohydrate, fat and alcohol.

Additional aids used for the determination of portion size and identification of food items included: the DAEK food photo cards and the DAEK generic life-size sketches of food portions as well as food models made according to the instructions in the DAEK manual.
A copy of the guidelines that were followed for carrying out the dietary intake assessment is in appendix 8.

2.14 Laboratory Methodology for Blood and Chemistry Tests

2.14.1 *Haematology and Biochemistry tests*

Complete blood count including haemoglobin (Hb), haematocrit (HCT), mean cell volume (MCV), platelet count (PL), white cell count (WCC) and lymphocyte count were done routinely at the NHLS laboratories.

Biochemistry tests included total protein (TP) and albumin (Alb); these were also done routinely at the NHLS laboratories.

2.14.2 HIV DNA PCR

HIV DNA PCR was done routinely at six weeks and at 9 months; if positive it was repeated at the next visit and also done earlier if the child looked symptomatic. These were done as per national guidelines at the NHLS laboratories.

2.14.3 CD4 count

CD4 lymphocyte counts were measured by flow-cytometry using the FACSCalibur (Becton Dickinson, San Jose, CA, USA). These were done routinely at the NHLS laboratories every six months as per national guidelines.

2.14.4 Micronutrient analysis of plasma and breastmilk samples

Whole breastmilk samples were aliquoted into 5ml labeled cryotubes and stored at -70◦C. These were then transported at the end of the study to NIRU (Nutrition Intervention research unit) MRC, Cape Town for analysis.

Blood samples were collected in metal free plain tubes, EDTA tubes and plain clot activator tubes; these were centrifuged at 5000 G for 10 minutes at -20◦C and the serum was aliquoted into labeled cryotubes and stored at -70◦C during the course of the study. They were then transported to NIRU MRC, Cape Town for analysis at the end of the study.

Zinc: A Thermo Elemental Atomic Absorption Spectrophotometer – Solaar S Series was used to assess the Zinc concentration in serum and milk samples. The method used was flame atomic absorption spectrophotometry (FAAS). The coefficient of variation for the inter batch precision was 2% and for the intra batch precision was 6%.

Vitamin A: Retinol levels in breastmilk were determined by using a reversed-phase high-performance liquid chromatographic (HPLC) method with wavelength-programmed ultraviolet-visible absorbance detection. The coefficient of variation for the inter batch precision was 2.5% and for the intra batch precision was 3%. The methodology described by Tanumihardjo and Penniston (2002) was used for breastmilk retinol analysis.

Vitamin B12 and Folate: For the simultaneous quantitative determination of Vitamin B12 and Folate in serum and plasma, PerkinElmar Wizard automatic gamma counter was used (SimulTRAC-SNB Cat no. 06B257117). The coefficient of variation for the inter batch precision was 7% and for the intra batch precision was 9%.

2.15 Breast milk macronutrient analysis

Analyses were done using a Miris® Human milk Analyser which uses mid spectra infrared spectroscopy to measure the fat, protein, lactose and solids content of the breastmilk. The energy content is calculated from the fat, protein, lactose and solids content in the breastmilk.

Instrument specifications: Miris® Human milk Analyser, HMA, Miris AB, Sweden, **www.miris.se**. This instrument was originally developed to analyse cow's milk but was modified and calibrated for human milk. The instrument has been used in a previous published study (Menjo et al, 2009). Menjo et al validated the instrument against conventional methods of analysis and found that there was a significant correlation between the two methods ($p<0.001$); in fact they managed to reduce the volume of milk used to 0.5ml by diluting it and getting accurate results for protein and fat content but not for lactose content in the diluted samples. The r value obtained by the investigators between the conventional method for fat estimation (Folch) and the HMA was 0.93; between the conventional method of protein estimation (Kjedahl) and the HMA was 0.92; and between the conventional method for lactose estimation (HPLC) and the HMA was 0.72.

Procedure: Frozen whole breast milk samples collected over the duration of the study were thawed and warmed to around 36-40º C. Following which the sample was homogenized using an ultrasonic processor VCX 130.

The instrument was calibrated using 'Miris Check' before analysis. The instrument has two modes-homogenized milk and non homogenized milk. The homogenized mode was chosen. Following this, 2 mL of the homogenized sample were injected into the inlet. Readings were made in duplicate and the mean calculated was used.

Figure 5: The Miris Human milk Analyser

2.16 Cervical smear

Cervical smears were done at six months using an endocervical brush during a pelvic examination. The slide was fixed and labeled with the patient ID and sent to the NHLS laboratories for analysis.

2.17 Data preparation

All clinical, laboratory, anthropometric and diet data was entered into an MS Access (MS Office Access 2003) database created for the study. The data was rechecked for any missing data and entry errors. Mothers and infants were identified by a unique study ID to maintain confidentiality; although only the principal investigator had access to the database. Mothers and their infants were linked through the study ID.

Specific queries related to the study objectives were created in MS Access. These queries were then exported through to SPSS.

Certain variables were categorized to study associations/trends within categories. BMI was categorized as ≤24.9 kg/m2 and ≥25 kg/m2. An MUAC measurement less than 23cm was considered to be associated with wasting (Gartner et al, 2001).

Duration of breastfeeding was categorized to ≤ 3.5 months and > 3.5 months. Disease progression was categorized as progression to WHO stage 3 or higher; a poor Karnofsky score was categorized as 80% or below; and an SRQ20 score of 8 and above is normally associated with depression and it was therefore categorized as such for this analysis. Besides this all continuous variables were assessed against normal values (appendix 9).

2.18 ***Statistical methods***

SPSS 15.0 for windows was the software used for analysis (© SPSS Inc.). Data from MS Access 2003 was exported into an SPSS datasheet.

To assess for the impact of breastfeeding on maternal and infant nutition and health, the formula feeding group and the breastfeeding control groups were compared.

To assess for the impact of nutritional supplementation on maternal and infant nutrition and health, the breastfeeding control and supplemented groups were compared.

Socio-demographic variables and laboratory parameters viz. CD4, haemoglobin and albumin were analysed to check for any differences between the groups at baseline. Paired T tests for normally distributed variables and Wilcoxon signed rank test for skewed variables were performed to study changes over time in the laboratory parameters, anthropometric and body composition measurements.

Continuous variables were checked for normality of distribution by performing a one sample Kolmogorov-Smirnov test. This test was used as it is an objective test and a significant p value signifies that the distribution is skewed or not normal, whereas the histogram was used to subjectively assess distribution and direction of skewing. Logarithmic transformation was done for the continuous variables where the distribution was skewed. Student t tests and Independent t tests were done for the continuous variables. Non parametric tests were conducted for those continuous variables which could not be transformed and medians with their inter-quartile range are reported.

Differences between the variables over time were calculated and these were then compared between groups to answer the study questions viz: the impact of breastfeeding using the formula feeding group as control and the impact of the nutritional supplement to breastfeeding mothers using the breastfeeding control group as control. The analysis was conducted using Independent T tests for the normally distributed variables and the Mann-Whitney tests for the skewed variables.

Incidence rates and incidence rate ratios were calculated for clinical events, opportunistic infections, worsening of WHO stage, Karnofsky score and SRQ 20 score using STATA 9.2 version ©StataCorp (4905 Lakeway drive, College station, Texas 77845 USA; http://www.stata.com).

As both breastfeeding and a low BMI have in previous studies been proposed to be important prognostic markers for breastfeeding HIV positive women, all parameters where possible were analysed as such; per duration of breastfeeding; and per BMI category in the breastfeeding groups.

As the data was longitudinally collected, an intention to treat analysis to include all the subjects (the ones who may have missed visits) generalized estimating equations with robust estimators and an exchangeable covariance matrix were used to analyse the continuous variables over time. Generalised linear models with Poisson distribution and log function were used to analyse the incidence rates of the opportunistic infections.

All child growth data including gender, birth date and date of observation was entered into a spreadsheet and the WHO *igrowup* SPSS syntax was run to calculate age and sex appropriate

z scores for weight for age; length for age; head circumference for age; weight for length; MUAC for age; TSF for age; and BMI for age (WHO, 2009a).

Chapter 3

Results

During the period December 2006 to July 2008 we enrolled 220 mothers of which 204 were eligible for analysis as they fulfilled the eligibility criteria namely completing at least 2 study visits. The study follow-up was complete by May 2009. Of the 204 mother -baby pairs on follow-up, 86% completed all study visits.

3.1 Baseline socio-demographic and laboratory parameters

Socio-demographic data was available for all 204 subjects. Of the 204 subjects, 75 were in the formula feeding group (Group1); 63 in the breastfeeding control group (Group 2) and 66 in the breastfeeding supplemented group (Group 3).

Formula feeding and breastfeeding control groups (table 1a)

On assessing for differences between the formula feeding and breastfeeding control groups, the breastfeeding mothers were significantly younger when compared to the formula feeding mothers (p=0.002). Most of the mothers were unmarried (93.7-94.7%); living with family or a partner.

Although more of the mothers in Group 1 (64%) had access to piped water at home, the difference was not statistically significant; access to electricity and waterborne sanitation were also not significantly different between the 2 groups. Around 31 % of the mothers reported experiencing household food insecurity; however there were no significant differences between the two groups. There was trend towards significance for more formula feeding mothers (36%) to be self-reliant for their source of income. The median schooling was between 11-12 years in the two groups; and median household size was 3 (IQR 2-5); there

were no statistically significant differences between the two groups; for any of these variables. More mothers in the formula feeding group disclosed their HIV status to family and partner compared to those in the breastfeeding group, but this difference was not statistically significant. A similar number of mothers (72-76%) reported use of oral and injectable contraceptive methods; however use of condoms was not reported by any mothers. The reported number of viable pregnancies (parity) was similar in the two groups (Median=2). There were no significant differences for history of TB or STI. The two groups were similar in their mean BMI (27.04-27.81 kg/m^2) ; median CD4+ lymphocyte count (413-433 cells/mm3) ; mean haemoglobin (11.77-11.84 g/dL); mean serum total protein (79.12-80.3 g/L); and mean serum albumin fraction (32.27-32.86 g/dL). All mothers were WHO stage 1 in the two groups.

Breastfeeding supplemented and control groups (table 1b)

On assessing for differences between the two breastfeeding groups, both groups were similar and no significant differences were seen. There was a trend for mothers in the control group to have higher albumin levels, however it did not reach significance (0.065).
Randomisation was effective in producing two similar groups as there were no statistically significant differences between the supplemented and non supplemented breastfeeding groups in the parameters investigated.

Looking at the three groups of mothers as a whole, 93 (45.6%) had serum albumin levels below normal values (<32g/L) and 90 (44.1%) had haemoglobin levels below normal (<11.5g/dL); (figures 6 and 7, section 3.3).

Table 1a: Socio-demographic and laboratory parameters of the mothers at baseline in the formula-feeding and the breastfeeding control groups

Variables		Formula Feeding (n=75)	Breastfeeding (n=63)	p value for comparison between groups
Age years Mean (SD)		27.57 (5.51)	24.9 (4.21)	0.002
Marital status: Single/cohabiting (% within group)		71 (94.7)	59 (93.7)	0.799
Access to water (% within group)	**Own tap**	48(64)	36 (57.1)	0.427
	Public tap	27 (36)	26 (41.3)	
	Other	0 (0)	1(1.6)	
Access to Electricity (% within group)		44 (58.7)	38 (60.3)	0.844
Access to Sanitation (% within group)	**Waterborne**	42 (56)	29 (46)	0.506
	Pit	32 (42.7)	33 (52.4)	
	Nil	1 (1.3)	1 (1.6)	
Source of income (%within group)	**Self**	27 (36)	12 (19)	0.058
	Partner	25 (33.3)	25 (39.7)	
	Family	22 (29.3)	21 (33.3)	
	Grants	1 (1.3)	5 (7.9)	
Food insecurity (% within group)		23 (30.7)	20 (31.7)	0.892
Disclosure to family (% within group)		43 (57.3)	29 (46)	0.128
Disclosure to partner (% within group)		53 (70.7)	38 (60.3)	0.175
Contraception use (% within group)		54 (72)	48 (76.2)	0.577
Median years of schooling (IQR)		12 (10-12)	11 (10-12)	0.588
Median size of household (IQR)		3 (2-5)	3 (2-5)	0.832
Median parity (IQR)		2 (1-3)	2 (1-3)	0.627
Past history of TB (% within group)		3 (4)	1 (1.6)	0.391
Past history of STI (% within group)		13 (17.3)	10 (15.9)	0.539
BMI kg/m^2 Mean (SD)		27.81 (5.39)	27.04 (4.27)	0.363
CD4 cells/µL Median(IQR)		433 (316-574)	413 (312-492)	0.622
Haemoglobin g/dL Mean (SD)		11.77 (1.6)	11.84 (1.59)	0.800
Total Protein g/L Mean (SD)		80.3 (7.73)	79.12 (7.12)	0.379
Albumin g/L Mean (95% CI)		32.86 (4.48)	32.27 (4.3)	0.454

Table 1b: Socio-demographic and laboratory parameters of the mothers at baseline in the breastfeeding control and supplemented groups

Variables		Breastfeeding: control (n=63)	Breastfeeding: supplemented (n=66)	p value for the difference between groups
Age years Mean (SD)		24.9 (4.21)	25.85 (4.85)	0.241
Marital status: Single/cohabiting (% within group)		59 (93.7)	65 (98.5)	0.155
Access to water (% within group)	**Own tap**	36 (57.1)	32 (48.5)	0.327
	Public tap	26 (41.3)	34 (51.5)	
	Other	1(1.6)	0 (0)	
Access to Electricity (% within group)		38 (60.3)	30 (45.5)	0.091
Access to Sanitation (% within group)	**Waterborne**	29 (46)	28 (42.4)	0.522
	Pit	33 (52.4)	38 (57.6)	
	Nil	1 (1.6)	0 (0)	
Source of income (%within group)	**Self**	12 (19)	16 (24.2)	0.286
	Partner	25 (39.7)	23 (34.8)	
	Family	21 (33.3)	26 (39.4)	
	Grants	5 (7.9)	1 (1.5)	
Food insecurity (% within group)		20 (31.7)	21 (31.8)	0.993
Disclosure to family (% within group)		29 (46)	27 (40.9)	0.557
Disclosure to partner (% within group)		38 (60.3)	40 (60.6)	0.973
Contraception use (% within group)		48 (76.2)	46 (69.7)	0.407
Median years of schooling (IQR)		11 (10-12)	11 (10-12)	0.515
Median size of household (IQR)		3 (2-5)	3 (2-5)	0.901
Median parity (IQR)		2 (1-3)	2 (1-3)	0.829
Past history of TB (% within group)		1 (1.6)	3 (4.5)	0.375
Past history of STI (% within group)		10 (15.9)	10 (15.2)	0.583
BMI kg/m^2 Median (IQR)		26.53 (23.37-30.1)	26.18 (23-30.67)	0.793
CD4 cells/µL Median(IQR)		413 (312-492)	443.5 (294.8-564)	0.658
Haemoglobin g/dL Mean (SD)		11.84 (1.59)	11.47 (1.52)	0.183
Total Protein g/L Mean (SD)		79.12 (7.12)	79.45 (8.22)	0.808
Albumin g/L Mean (SD)		32.27 (4.21)	30.86 (4.85)	0.065

3.2 PMTCT prophylaxis, delivery, and vertical transmission (table 2 a & b)

3.2.1 PMTCT prophylaxis

Antepartum prophylaxis with antiretrovirals for prevention of mother to child transmission (PMTCT) was received by 200 (98%) of the mothers. The regimens used were a single dose Nevirapine (sdNVP) alone (27.5%) or a combination of sdNVP and Combivir (47.1%). 193 (94.6%) of the babies received PMTCT prophylaxis (sdNVP).

3.2.2 Delivery details

Most mothers (63.7%) had a normal vaginal delivery; although caesarian sections were also frequent (36.3%). Indications for caesarian sections varied from 22(29.7%) for previous caesarean section (CS); 15 (20.3%) for foetal distress; 13 (17.6%) due to cephalo-pelvic disproportion and 11 (14.9%) due to poor progress of labour. Other reasons included ante-partum haemorrhage (6.8%); pregnancy induced hypertension (4.1%); breech (2.7%); and 1.4% each due to vulval warts, tubal ligation and multiple pregnancy. There were no significant differences between the mean birth weight in the three groups (p=0.778).

3.2.3 Vertical transmission

Vertical transmission by 6 weeks (in-utero/intra-partum): 14 infants (6.9%) tested HIV positive at 6/52 of age; 3 in the formula fed group; 5 in the breastfed control; and 6 in the breastfed supplemented group.

Vertical transmission by 14 weeks (late postnatal): One child in the breastfed supplemented group tested positive.

Vertical transmission by 36 weeks (late postnatal): One further child in the breastfed supplemented group tested positive.

Within each maternal prophylaxis category; 12.4% of the babies whose mothers had received sdNVP and 2.9% of the babies whose mothers had received sdNVP and Combivir were positive at six weeks suggesting intra-partum or in-utero infection.

Independent t tests and chi-squared tests were used to assess the risk factors for intra-partum and in-utero transmission such as low CD4 count; haemoglobin; albumin; BMI; mode of delivery; duration of rupture of membranes, episiotomy; birth weight; and antiretroviral prophylaxis for any significant associations with a positive HIV DNA PCR at 6 weeks. Albumin; type of maternal prophylaxis; and birth weight were found to be significantly associated with vertical transmission; p=0.017; p=0.019; and p=0.031 respectively. There was a trend towards significance for having had an episiotomy or a tear requiring suturing and vertical transmission (p=0.075). No other variables were found to be significantly associated with vertical transmission.

Table 2a: Description of PMTCT prophylaxis and delivery parameters

Prophylaxis and delivery parameters		**Formula feeding (n=75)**	**Breastfeeding control (n=63)**	**Breastfeeding supplemented (n=66)**	**p value**
Type of delivery	**NVD (%)**	47 (62.7)	40(63.5)	43 (65.2)	0.953
	CS (%)	28(37.3)	23(36.5)	23 (34.8)	
Duration of rupture of membranes Median (min/max)		0 (0-17)	0 (0-19)	0 (0-28)	0.835
Episiotomy/tear requiring sutures (%)		15 (20)	13 (20.6)	13 (19.7)	0.991
Antepartum prophylaxis	**sd NVP (%)**	31 (41.3)	30 (47.6)	36 (54.5)	0.136
	sd NVP and Combivir* (%)	44 (58.7)	30 (47.6)	29 (43.9)	
	Nil (%)	0	3 (4.8)	1 (1.5)	
PMTCT Prophylaxis to baby	**sd NVP (%)**	72 (96)	59 (93.7)	62 (93.9)	0.796
	Nil (%)	3 (4)	4(6.3)	4 (6.1)	
Female child (%)		39 (52)	30 (47.6)	31 (47)	0.808
Birth weight kg Mean (SD)		3.14 (0.38)	3.17 (0.46)	3.12 (0.44)	0.778
Vertical transmission (six weeks)		3 (4)	5 (7.9)	6 (9.1)	0.452

***sd NVP given during labour and Combivir given from 36 weeks as per MTCT Plus program guidelines

Table 2b: Associations between vertical transmission and risk factors

Risk factors		Vertical transmission		p value with 95% CI
		No	Yes	
Age years Mean (SD)		26.3 (5.05)	24.87(4.62)	0.276 (-1.15, 4.01)
Haemoglobin g/dL Mean (SD)		11.68(1.55)	11.83 (1.84)	0.735 (-0.98, 0.69)
Albumin g/L Mean (SD)		32.21	29.4 (4.81)	0.017 (0.52.5.1)
CD4 cells/µL Median (IQR)		440.5 (312,568.5)	348 (289.5, 439.8)	0.080
Birth weight kg Mean (SD)		3.16 (0.43)	2.92 (0.35)	0.031 (0.02, 0.46)
Maternal prophylaxis (% within group)	**sdNVP**	84 (86.6)	13(13.4)	0.019
	sdNVP and Combivir	100 (97.1)	3 (2.9)	
	Nil	4 (2.1)	0	
Infant prophylaxis (% within group)	**sdNVP**	179 (92.7)	14 (7.3)	0.209
	Nil	9 (81.8)	2 (18.2)	
Type of delivery (% within group)	**NVD**	118 (90.8)	12 (9.2)	0.244
	LSCS	70 (94.6)	4 (5.4)	
Episiotomy (%within group)		35(85.4)	6 (14.6)	0.075
Duration of rupture of membranes hours Median (min/max)		0 (0-28)	0 (0-11.2)	0.215

3.3 Intra-group comparison of changes in haematology and chemistry parameters in mothers over six months (table 3)

Haemoglobin: All three groups showed an increase in the mean haemoglobin; this increase was not significant. Although haemoglobin levels improved, 34% still had levels less than the normal minimal level of 11.5 g/dL.

Haematocrit: The mean haematocrit increased in all three groups, although the increase was significant only in Group 2 (p=0.04). The majority of the mothers (60%) had low haematocrit (<37%) at 2/52 and at six months (55.6%).

Mean cell volume (MCV): The mean cell volume decreased significantly in all three groups (p<0.001). MCV was below normal minimal levels (<79.1fL) in 4.2% of the mothers at baseline and in 8.6% of the mothers at six months. It was above normal maximal levels (>98.9 fL) in 19% of the mothers at baseline; no mother had a raised MCV at six months.

Lymphocyte count (L): No significant changes over time were noted.

Figure 6: Histogram of Haemoglobin levels (g/dL) at baseline for cohort

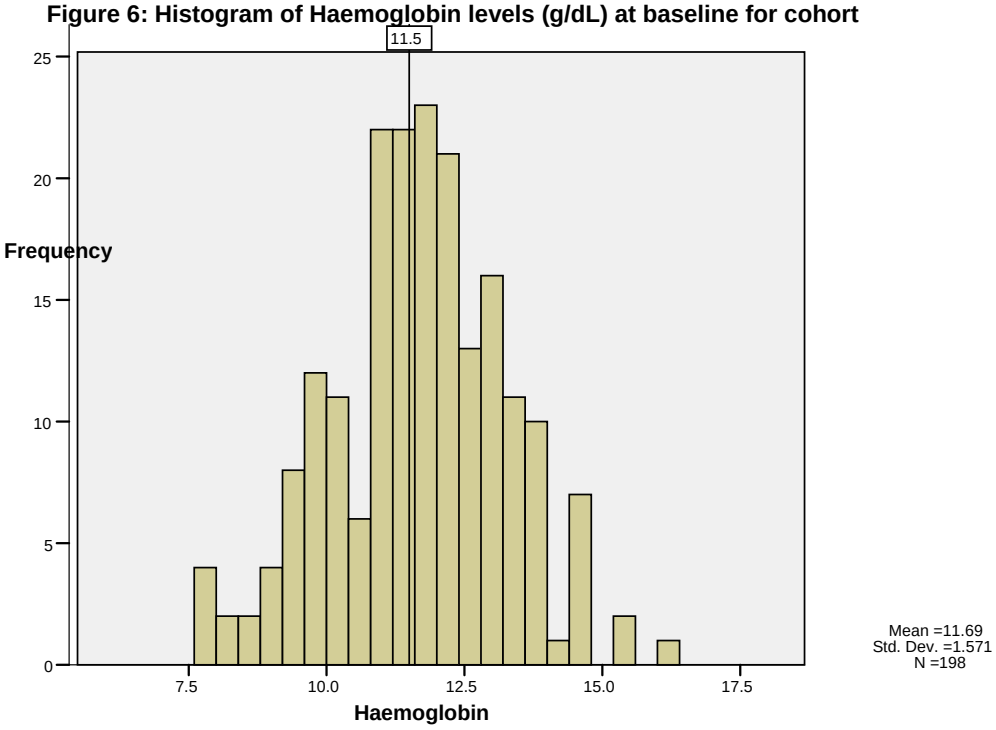
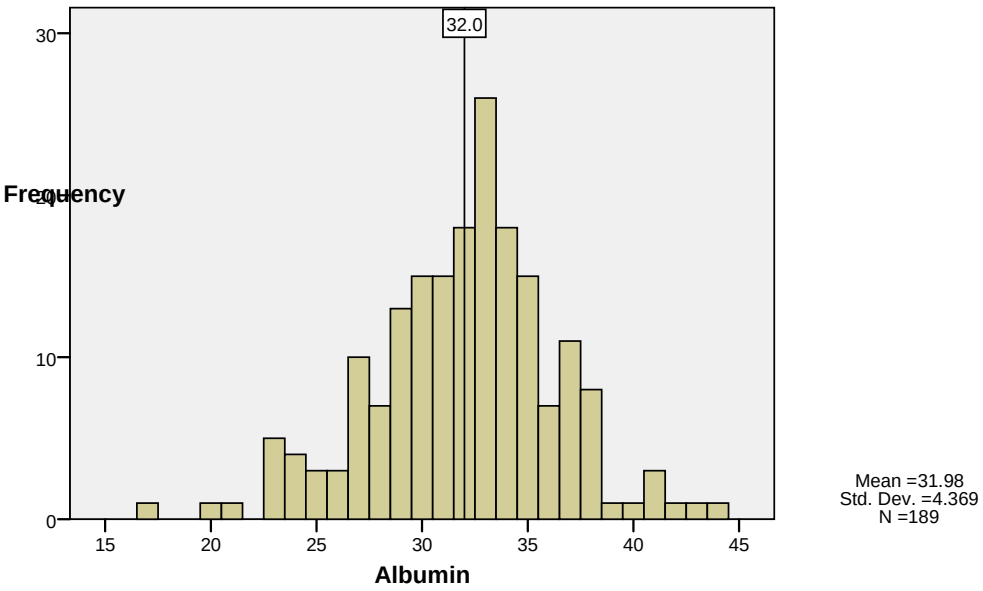


Figure 7: Histogram of Albumin (g/L) at baseline for the cohort



Total Protein: Increased in all three groups significantly ($p<0.001$).

Albumin: Increased in all three groups significantly ($p<0.001$). When assessed for low albumin levels, there were 11.8% mothers still with levels below the normal minimal level of 32 g/L.

Folate: Serum folate levels dropped in groups 1 and 2 and increased in group 3, although none of the changes were statistically significant. There were no mothers who had low levels of folate at baseline and only one at six months (deficiency defined as <3 ng/mL).

Vitamin B12: Vitamin B12 levels in the blood increased in all three groups, although only the increase in group 3 was significant ($p=0.024$).When assessed for B12 deficiency (<120 pg/mL) there were 2% mothers at baseline and at six months. At baseline and at six months 4.8% and 3 % of the mothers respectively had marginal levels (120 pg/mL ≤ B12 levels < 160pg/mL).

Zinc: Zinc levels also increased in all three groups, although only the increase in Groups 2 and 3 was significant at $p=0.008$ and $p=0.001$ respectively. More than half the mothers (67.6%) had low serum zinc levels (< 65 µg/mL) at baseline; but this improved to 50.5% at six months.

CD4: Median CD4 counts remained constant in groups 2 and 3 with a slight drop in group 1 which was not significant. Eight mothers had a drop in CD4 count to below 200 cells/µL by six months.

Table 3: Summary of intra-group changes in laboratory parameters over 6 months

Laboratory parameter	Formula feeding (n=57)			Breastfeeding: control(n=57)			Breastfeeding: supplemented(n=56)		
	2/52	6/12	p value for the change (95% CI)	2/52	6/12	p value for the change (95% CI)	2/52	6/12	p value for the change (95% CI)
Hb g/dL Mean (SD)	11.85 (1.7)	11.99 (1.3)	0.449 (-0.51, 0.23)	11.76 (1.49)	12.09 (1.22)	0.097 (-0.7, 0.06)	11.37 (1.47)	11.52 (1.0)	0.393 (-0.52, 0.21)
Haematocrit % Mean (SD)	36.07 (4.96)	36.93 (3.44)	0.153 (-2.07, 0.34)	35.52 (4.17)	36.57 (3.26)	0.04 (-2., -0.05)	34.88 (3.86)	35.33 (3.45)	0.371 (-1.43, 0.54)
MCV fL Mean (SD)	92.95 (8.27)	87.01 (6.85)	<0.001 (3.98, 7.91)	92.3 (6.43)	86.41 (4.26)	<0.001 (4.27,7.5)	92.43 (7.51)	85.93 (4.66)	<0.001 (4.66, 8.33)
Lymph x 10^9/ L Mean (SD)	2.20 (0.57)	2.26 (0.84)	0.642 (-0.35, 0.22)	2.11 (0.64)	2.20 (0.60)	0.367 (-0.3, 0.12)	2.17 (0.67)	2.16 (0.72)	0.985 (-0.16, 0.17)
Platelets x10^9/ L Mean (SD)	393.73 (136.6)	273.40 (78.8)	<0.001 (80.2, 160.4)	385.02 (105.5)	284.27 (67.32)	<0.001 (73.5, 128)	389.76 (123.74)	279.44 (78.34)	<0.001 (77, 143.7)
Total Protein g/L Mean (SD)	80.44 (8.1)	86.96 (11.1)	<0.001 (-9.13, -3.91)	78.8 (7.18)	86.96 (9.35)	<0.001 (-10.6, -5.8)	79.51 (8.29)	86.42 (9.92)	<0.001 (-8.9, -4.91)
Albumin g/L Mean (SD)	32.92 (4.42)	37.1 (3.93)	<0.001 (-5.45, -2.93)	32.5 (4.4)	36.46 (4.6)	<0.001 (-5.5, -2.46)	30.51 (4.38)	35.3 (4.5)	<0.001 (-5.74, -3.84)
Folate ng/mL Mean (SD)	13.29 (4.23)	12.98 (4.29)	0.617 (-0.95, 1.58)	11.63 (3.09)	11.01 (4.2)	0.425 (-0.95, 2.2)	10.86 (5.23)	11.84 (5.22)	0.413 (-3.38, 1.43)
B12 pg/ dL Med (IQR)	286.5 (208.3-372.3)	290 (192-427)	0.666	263 (174-320)	291 (169-336)	0.219	285 (194-400)	334.5 (257.8-432)	0.024
Zinc µg/mL Mean (SD)	64.32 (15.02)	66.63 (13.11)	0.376 (-7.55, 2.93)	59.18 (11.93)	64.17 (10.98)	0.008 (-8.6, -1.4)	57.5 (9.65)	62.31 (12.65)	0.001 (-7.49, -2.14)
CD4 cells/µL Med (IQR)	432.5 (315-577.8)	424 (305.5-577)	0.208	405.5 (311-498)	405 (311-519)	0.554	405.5 (311-498)	405 (310.5-519)	0.987

3.4 Changes in anthropometric and body composition measures in mothers over 6 months: intra-group comparison (table 4)

Weight: Median weight decreased in all three groups, but the decrease was statistically significant only in groups 1 and 3; p value of 0.045 and 0.022 respectively.

Body mass index (BMI): The median BMI also significantly decreased in groups 1 and 3 (see figure 8). One mother in the formula feeding group had a BMI of 17.48kg/m^2 (underweight category as per WHO guidelines, 2006) and 8 mothers had a BMI of less than 20 kg/m^2 (>18.5 kg/m^2) at six months.

Triceps skin fold thickness (TSF): TSF also increased significantly in all three groups (figure 9).

Mid-upper arm circumference (MUAC): The median MUAC measurements increased significantly in all three groups (see figure 10). We found that four mothers had MUAC <23 cm at baseline and by six months, this number had reduced to three.

Lean Body Mass (LBM): The mean lean body mass decreased significantly in all three groups (see figure11).

Fat Mass (FM) and%FM: The median fat mass and percentage fat increased significantly in all three groups (see figure 12 and 13).

Table 4: Summary of changes in anthropometry and body composition measures over 6 months: intra-group changes

Measurements	Formula feeding (n=60)			Breastfeeding: control (n=57)			Breastfeeding: supplemented (n=56)		
	2/52	6/12	p value for the change (95% CI)	2/52	6/12	p value for the change (95% CI)	2/52	6/12	p value for the change (95% CI)
Weight kg Median (IQR)	65.88 (59.28-77.21)	64.5 (58.35-77.63)	0.045	66.05 (57.5-71.85)	65.15 (56.93-73.7)	0.397	65.45 (59.1-75.25)	63.13 (56.4-72.58)	0.022
BMI kg/m^2 Median (IQR)	26.81 (23.79-31.02)	25.51 (23.29-31.28)	0.045	26.52 (23.32-30.02)	26.84 (23.18-30.18)	0.492	26.18 (23.01-30.67)	25.04 (22.51-30.68)	0.028
MUAC cm Median (IQR)	28.08 (25.93-31.23)	28.5 (26.75-32.65)	0.002	28 (26.15-29.48)	29.3 (27-31.7)	<0.001	27.6 (25.98-30.5)	28.5 (25.91-31.6)	0.001
TSF mm Median (IQR)	15.38 (12.7-22.3)	20.75 (15.25-26)	<0.001	16.5 (13.44-20.38)	20 (17-26)	<0.001	16 (14-18.78)	18 (15-25)	<0.001
LBM kg Mean (SD)	46.64 (6.77)	42.66 (5.01)	<0.001 (2.5, 5.4)	45.23 (6.12)	41.46 (5.4, 0.96)	<0.001 (2.3, 5.3)	45.72 (5.8)	42.51 (4.63)	<0.001 (1.81, 4.59)
Fat Mass kg Median (IQR)	20.46 (15.63-29.27)	24.25 (18.54-33.53)	0.019	19.88 (16.12-27.59)	24.91 (18.72-29.07)	<0.001	20.73 (15.8-26.1)	25.85 (19.51-32.19)	0.014
% Fat Mass Mean (SD)	30.83 (7.84)	35.3 (8.11)	0.001 (-7, -2)	30.8 (8.15)	36.87 (7.86)	<0.001 (-8.0, -4.1)	32.05 (5.38)	36.54 (5.8)	<0.001 (-7.08, -1.91)

Figure 8: Mean and 95% CI of BMI over time

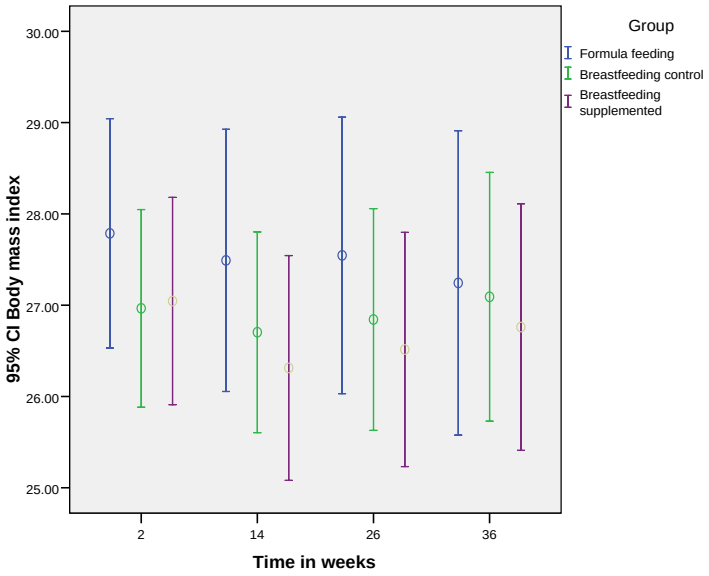


Figure 9: Mean and 95% CI of TSF over time

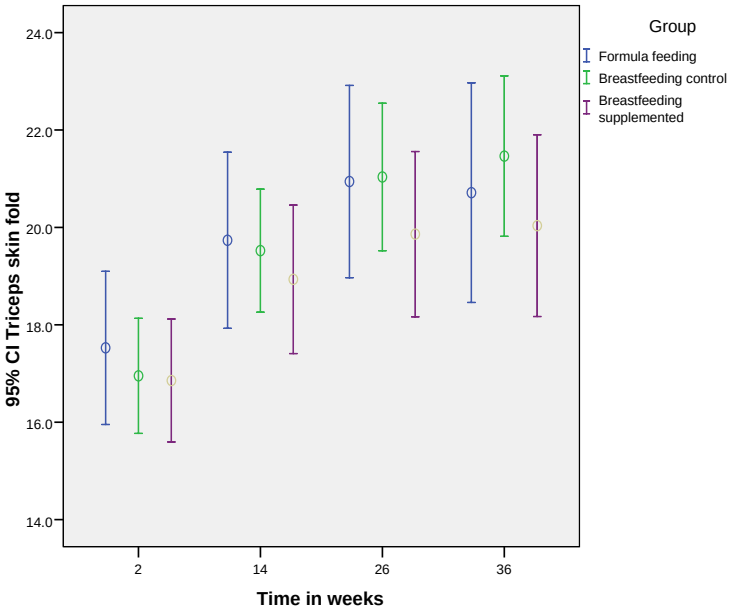


Figure 10: Mean and 95% CI of MUAC over time

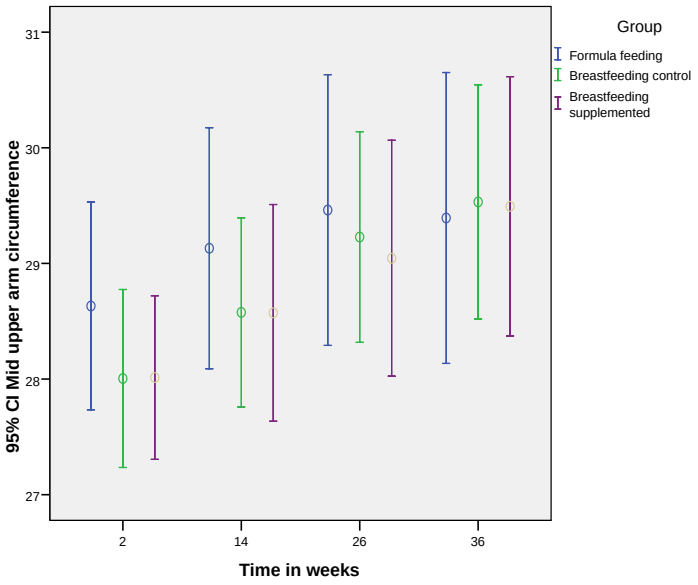


Figure 11: Mean and 95% CI of LBM over time

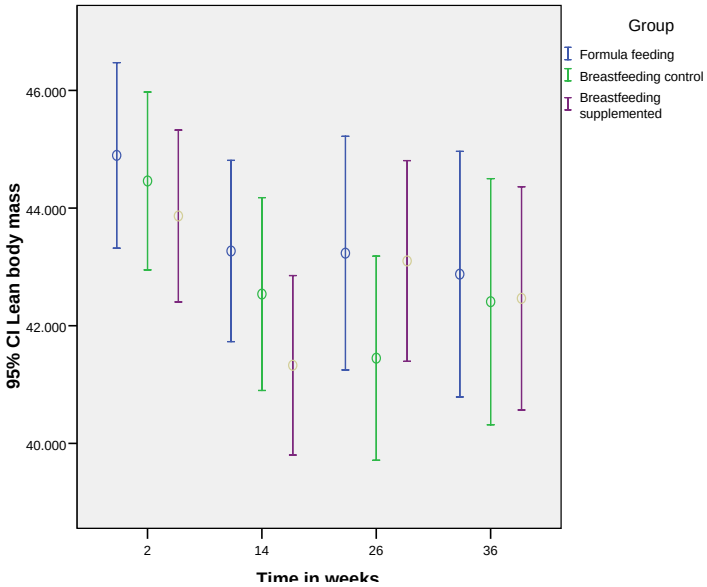


Figure 12: Mean and 95% CI of Fat mass over time

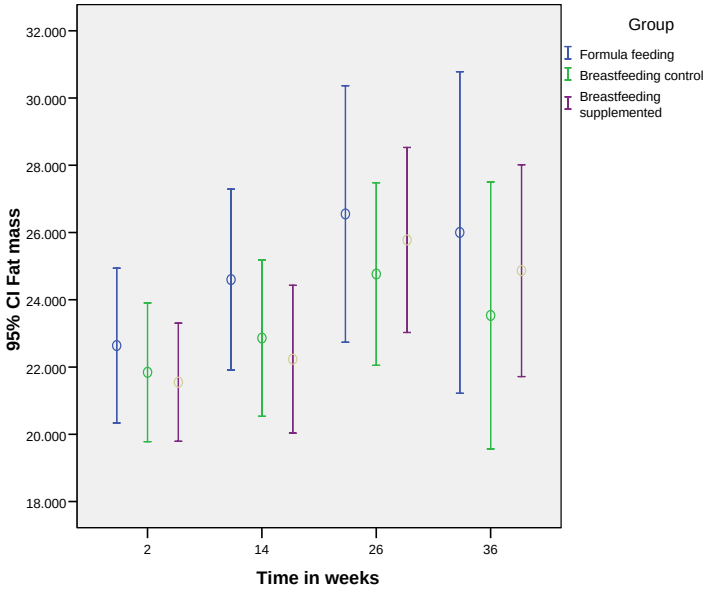
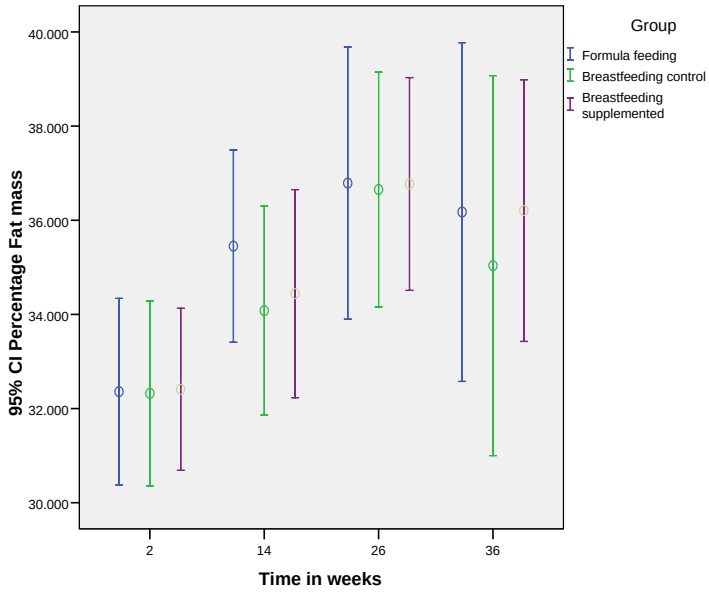


Figure 13: Mean and 95% CI for Percentage fat mass over time



3.5 Baseline anthropometric measures in all the infants

There were no significant differences between the three groups at baseline.

None of the infants fell into the wasted or stunted categories (z score less than -2 standard deviations of the reference); (table 5). Z score for the head circumference for age was surprisingly above the reference range.

Table 5: Baseline anthropometric measures in the infants at 2 weeks of age

	Formula feeding (n=74)	**Breastfeeding control (n=63)**	**Breastfeeding supplemented (n=65)**	**p value for the difference between the groups**
Weight kg Mean (SD)	3.62 (0.45)	3.72 (0.58)	3.66 (0.48)	0.492
Length cm Mean (SD)	51.05 (1.92)	50.89 (2.42)	51.02 (1.98)	0.890
HC cm Mean (SD)	36.29 (1.33)	36.2 (1.43)	36.45 (1.19)	0.550
BMI kg/m^2 Mean (SD)	13.83 (1.2)	14.27 (1.49)	14.03 (1.35)	0.159
Weight/Age z scores Mean (SD)	-0.36 (0.9)	-0.14 (1.05)	-0.21 (0.89)	0.396
Length/Age z score Mean (SD)	-0.66(1.03)	-0.72 (1.12)	-0.63 (1.02)	0.898
HC /Age z score Mean (SD)	0.42 (1.09)	0.37 (1.06)	0.60 (1)	0.418
BMI/Age z score Mean (SD)	0.005 (0.9)	0.37 (1.09)	0.2 (1)	0.103
Weight/Length z score Mean (SD)	0.05 (0.98)	0.46 (1.07)	0.2 (1.13)	0.080

3.6 Effect of breastfeeding on maternal and infant health

3.6.1 Effect of breastfeeding on anthropometric and body composition measures

3.6.1.1 Cross-sectional analyses at 14 weeks post delivery

There were no significant differences between the two groups at 14 weeks (table 6a).

Table 6a: Effect of breastfeeding on change in body composition and anthropometric measures at 14 weeks

Change in parameters from 2 weeks to 14 weeks	**Formula feeding (n=68)**	**Breastfeeding Control (n=60)**	**p value for the difference between the groups (95% CI)**
Weight in kg Mean (SD)	-1.29	-0.50	0.383 (-1.89, 0.31)
BMI in kg/m^2 Mean (SD)	-0.51	-0.18	0.445 (-0.78, 0.11)
MUAC in cm Mean (SD)	0.34	0.68	0.420 (-0.73, 0.07)
TSF in mm Mean (SD)	2.07	2.69	0.514 (-1.79, 0.55)
LBM in kg Mean (SD)	-2.83	-1.99	0.283 (-2.12,0.44)
Fat mass in kg Mean (SD)	1.24	1.87	0.624 (-2.17, 0.90)
%Fat mass Mean (SD)	2.45	2.98	0.541(-2.42,1.35)

3.6.1.2 Cross-sectional analysis at 6 months post delivery (table 6b)

There were no significant differences between the changes in both groups. Both groups lost weight, BMI and LBM from two weeks to six months; and increased in MUAC, TSF, fat mass and percentage fat mass measurements.

Table 6b: Effect of breastfeeding on change in body composition and anthropometric measures at 6 months

Change in parameters from 2 weeks to 6 months	Formula feeding (n=59)	Breastfeeding Control (n=56)	p value for the difference between the groups (95% CI)
Weight in kg Mean (SD)	-1.15 (4.47)	-0.43 (4.78)	0.407 (-2.41, 0.98)
BMI in kg/m^2 Mean (SD)	-0.44 (1.8)	-0.13 (1.94)	0.367 (-1, 0.37)
MUAC in cm Mean (SD)	0.71 (1.47)	1.20 (1.62)	0.087 (-1.06, 0.07)
TSF in mm Mean (SD)	3.09 (4.16)	4.14 (4.69)	0.200 (-2.68, 0.57)
LBM in kg Mean (SD)	-4.07 (3.6)	-3.78 (4.1)	0.782 (-2.37, 1.79)
Fat mass in kg Mean (SD)	3.0 (5.53)	4.14 (4.57)	0.395 (-3.83,1.53)
%Fat mass Mean (SD)	4.63 (6.20)	6.07 (5.46)	0.353 (-4.55, 1.65)

3.6.1.3 Longitudinal analyses using GEE in an exchangeable covariance matrix:

We performed an intention to treat analysis using generalized estimating equations in an exchangeable covariance matrix with robust errors. There were no significant differences between the groups for any of the anthropometric and body composition measurements over nine months of follow-up on adjusting for baseline age and CD4 count. Older mothers had a significantly higher weight, BMI, fat mass, MUAC and TSF. Mothers with higher CD4 counts also had significantly higher TSF and MUAC measurements (table 6 c to i; appendix 10).

3.6.2 Effect of breastfeeding on basic laboratory parameters at 6 months in the mothers **(table 7)**

There were no significant differences between the changes in both groups. Haemoglobin, haematocrit, lymphocyte count, total protein and serum albumin increased in both groups from baseline to 6 months. MCV; platelet, white cell and CD4 + lymphocyte count dropped in both groups of mothers from baseline to six months.

Table 7: Effect of breastfeeding on changes in laboratory parameters at 6 months in the mothers

Change in variable over 6 months	**Formula feeding (n=56)**	**Breastfeeding: Control (n=56)**	**p value for the difference between the groups (95% CI of the difference)**
Haemoglobin g/dL Mean (SD)	0.11 (1.33)	0.33 (1.41)	0.423 (-0.75, 0.32)
Haematocrit % Mean (SD)	0.75 (4.02)	1.04 (3.5)	0.705 (-1.82, 1.24)
MCV fl Mean (SD)	-5.73 (6.53)	-5.89 (5.7)	0.901 (- 2.34, 2.65)
Platelet count x 10^9/L Mean (SD)	-119.83 (139.5)	-100.75 (96.76)	0.430 (-66.92, 28.75)
Lymphocyte count x 10^9/L Mean (SD)	0.06 (0.90)	0.1 (0.7)	0.822 (-0.310, 0.389)
Total Protein g/L Mean (SD)	6.43 (9.06)	8.16 (8.36)	0.331 (-5.27, 1.79)
Albumin g/L Mean (SD)	4.26 (4.36)	3.96 (5.26)	0.765 (-1.66, 2.25)
White cell count x 10^9/L Median (IQR)	-0.6 (-1.6,0.7)	-0.40 (-1.7,0.40)	0.662
CD4 cells/µL Median (IQR)	-8 (-138,60)	-8 (-89.5, 73.5)	0.636

3.6.3 Effect of breastfeeding on changes in selected micronutrients from baseline to 6 months (table 8)

There were no significant differences for any of the changes between the two groups. Serum folate levels dropped and serum zinc levels increased in both groups. B12 levels dropped in the formula feeding group by15.61 pg/mL and increased by 34.17 pg/mL in the breastfeeding control group; however the difference between the groups was not statistically significant.

Table 8: Effect of breastfeeding on changes in selected micronutrients at 6 months

Mean change in variable over 6 months (sd)	Formula feeding (n=30)	Breastfeeding Control (n=30)	P value for the difference between the groups (95% CI)
Folate ng/ml	-0.31 (3.44)	0.62 (4.57)	0.759 (-1.7, 2.32)
Zinc µg/ml	2.31 (14.28)	4.99 (10.63)	0.383 (-8.77, 3.41)
B12 pg/ml	-15.61 (139.79)	34.17 (127.09)	0.135 (-115.41, 15.84)

3.6.4 Effect of breastfeeding on disease progression, quality of life and depression scores

3.6.4.1 Crude analysis

There were no significant differences in the crude incidence rates of clinical events or scores in the formula feeding group compared to the breastfeeding control group; (see table 9a). One subject in Group 1 was diagnosed with TB. The formula feeding group compared to the breastfeeding group had more women progressing to WHO stage 3 (4 vs. 0). More formula feeding women compared to breastfeeding women (16 vs. 6) had SRQ scores of ≥ 8; which is the WHO cutoff of an indicator for depression. This was evidenced by an incidence rate ratio of 0.46 when the breastfeeding mothers were compared to the formula feeding mothers; although the difference did not reach significance.

Table 9a: Effect of breastfeeding on disease progression, quality of life and depression score: Crude incidence rates and incidence rate ratios of various opportunistic and other clinical events per 1000 person weeks of follow-up

Clinical events	Formula-feeding (n=75)		Breastfeeding control (n=63)		Incidence rate ratio (95% CI)	p value
	Events	Crude incidence rate	Events	Crude incidence rate		
Oral thrush	4	1.81	1	0.56	0.31 (0.04-2.44)	0.310
PID	12	5.42	8	4.45	0.82 (0.34-2.01)	0.678
LRTI	5	1.36	3	2.78	2.05 (0.51-8.33)	0.344
History of Admissions	1	0.45	1	0.56	1.23 (0.08-19.59)	0.896
Breast pathology	1	0.45	5	2.78	6.16 (0.94-40.26)	0.076
Genital tract infections	54	24.39	32	17.8	0.73 (0.47-1.13)	0.157
TB infection	1	0.45	0	0	0	0.552
Herpes simplex	2	0.9	1	0.56	0.62 (0.06-6.63)	0.745
SRQ score ≥ 8	16	7.23	6	3.34	0.46 (0.18-1.15)	0.101
Karnofsky score ≤ 80	2	0.9	1	0.56	0.62 (0.06-6.63)	0.745

3.6.4.2 Longitudinal analyses using Poisson distribution analyses in a generalized linear model(GLM)

As the data was longitudinally collected, we did an intention to treat analysis adjusted for the *a priori* confounders: baseline age, CD4 and BMI; using generalized linear model with a Poisson distribution and a log function.

There were no significant differences between groups for the incidence rates of all events of opportunistic infections on adjusting for age and CD4 count except for the breastfeeding control group having lower depression scores i.e.fewer events of SRQ20 scores being ≥8; (p=0.043); (table 9 b-g appendix 11)

3.6.5 Effect of breast feeding on growth and development of the infant

3.6.5.1 Cross-sectional analyses

Growth and development at 14 weeks: The breastfeeding control group did significantly better in terms of growth; with significantly higher mean measurements for all parameters except length and head circumference and their z scores (see table 10a). Z scores for weight for age were 2 standard deviations below the reference (classified as underweight) for 3.9% of the children at 14 weeks; (see figure 14). Z scores for length for age were 2 standard deviations below the reference range (classified as stunted) in 9.4% of the children; (see figure 15). There was only one child (formula feeding) with a mean MUAC/ age z score which was 2 standard deviations below the reference range (classified as wasted); (see figure 16). There were no children with weight/length z scores 2 standard deviations below the reference (see figure 17). Although the median truncated Denver development score was similar in the two

groups, a significantly larger number of infants in the formula fed group had poorer scores (p=0.03; Mann Whitney U test).

Growth and development at 6 months: There were no significant differences in any of the growth parameters or the truncated Denver development score to assess the motor development (see table 10b). There was one child in the breastfeeding group who was underweight at six months. Z score for length for age fell in the stunted category for 5.1% of the children at six months. One child in the formula feeding group had weight/length z score 2 standard deviations below the reference. Z scores for MUAC for age were below 2 standard deviations of the reference for 2.5% of the children.

Growth and development at 9 months: There were no significant differences between the two groups for the growth parameters and the truncated Denver development score (see table 10c). One child in the breastfeeding group was underweight; six (5.6%) in both groups were stunted and 1 child was wasted in the formula-feeding group. There were two children in the formula feeding group with weight/length z score 2 standard deviations below the reference.

Table 10a: Effect of breastfeeding on growth and development of the infant at 14 weeks

Growth parameter	**Formula feeding (n=67)**	**Breastfeeding control (n=60)**	**P value for the difference between the groups**
Weight kg Mean (SD)	6.35 (0.81)	6.73 (0.89)	0.013 (-0.68, -0.08)
Length cm Mean (SD)	60.02 (2.29)	60.28 (2.62)	0.554 (-1.12, 0.6)
HC cm Mean (SD)	41.08 (1.47)	41.04 (1.51)	0.884 (-0.48, 0.56)
MUAC cm Mean(SD)	13.35 (1.16)	13.95 (1.09)	0.003 (-0.99,-0.2)
TSF mm Mean(SD)	10.15 (1.66)	10.93 (1.46)	0.006 (-1.33, -0.23)
BMI kg/m^2 Mean (SD)	17.57 (1.49)	18.45 (1.59)	0.002 (-1.42, -0.34)
Weight/ Age z scores Mean (SD)	-0.03 (1.04)	0.44 (1.03)	0.013 (-0.83, -0.1)
Length/ Age z score Mean (SD)	-0.69 (1.01)	-0.59 (1.08)	0.056 (-0.89, 0.01)
Head circumference/Age z score Mean (SD)	0.55 (1.1)	0.50 (1.07)	0.794 (-0.33, 0.43)
BMI/ Age z score Mean (SD)	0.50 (0.98)	1.06 (0.98)	0.002 (-0.9, -0.21)
Weight/ Length z score Mean (SD)	0.73 (1.11)	1.12 (1.05)	0.002 (-0.86, -0.2)
MUAC/ Age z score Mean (SD)	-0.06 (1.10)	0.49 (0.97)	0.003 (-0.93, -0.19)
TSF/ Age z score Mean (SD)	0.19 (0.99)	0.64 (0.77)	0.006 (-0.76, -0.13)
Truncated Denver development score Median(range)	4 (4-7)	4 (3-5)	0.03

Table 10b: Effect of breastfeeding on growth and development of the infant at 6 months

Growth parameter	Formula feeding (n=59)	Breastfeeding control (n=58)	p value for the difference between the groups (95% CI)
Weight kg Mean (SD)	7.99 (1.03)	8.35 (1.13)	0.078 (-0.75,0.04)
Length cm Mean (SD)	66.17 (2.2)	66.34 (2.54)	0.703 (-1.03, 0.7)
HC cm Mean (SD)	43.69 (1.58)	43.49 (1.64)	0.495 (-0.39, 0.79)
MUAC cm Mean (SD)	14.35 (1.37)	14.81 (1.26)	0.061 (-0.94, 0.02)
TSF mm Median(IQR)	10.75 (9-12)	10.88 (10-12.3)	0.239
BMI kg/m^2 Mean (SD)	18.22 (1.81)	18.9 (1.85)	0.045 (-1.36, -0.02)
Weight/Age z score Mean (SD)	0.31 (1.08)	0.61 (1.17)	0.153 (-0.71, 0.11)
Length/ Age z score Mean (SD)	-0.32 (0.97)	-0.35 (1.03)	0.898 (-0.34, 0.39)
HC/Age z score Mean (SD)	0.68 (1.2)	0.42 (1.19)	0.254 (-0.18, 0.69)
BMI/Age z score Mean (SD)	0.66 (1.13)	1.07 (1.14)	0.053 (-0.83, -0.01)
Weight/Length z score Mean (SD)	0.75 (1.12)	1.15 (1.11)	0.052 (-0.81, 0.004)
MUAC/Age z score Mean (SD)	0.24 (1.2)	0.62 (1.07)	0.074 (-0.79, 0.04)
TSF/Age z score Median(IQR)	0.86 (-0.04-1.44)	0.95 (0.48-1.6)	0.228
Truncated Denver development score Median(range)	3 (3-7)	3 (3-8)	0.692

Table 10c: Effect of breastfeeding on growth and development of the infant at 9 months

Growth parameter	Formula feeding (n=55)	Breastfeeding control (n=52)	p value for the difference between the groups
Weight kg Mean (SD)	9.06 (1.17)	9.42 (1.33)	0.148 (-0.83, 0.13)
Length cm Mean (SD)	70.49 (2.16)	70.63 (3.39)	0.805 (-1.22, 0.95)
HC cm Mean (SD)	45.11 (1.5)	45.07 (1.8)	0.899 (-0.59, 0.67)
MUAC cm Mean (SD)	14.95 (1.45)	15.3 (1.31)	0.190 (-0.88, 0.17)
TSF mm Mean (SD)	10.34 (2.08)	10.75 (2.09)	0.318 (-1.2, 0.39)
BMI kg/m^2 Mean (SD)	18.22 (1.92)	18.83 (1.93)	0.104 (-1.35, 0.13)
Weight/Age z scores Mean (SD)	0.38 (1.1)	0.65 (1.16)	0.220 (-0.7, 0.16)
Length/ Age z score Mean (SD)	-0.34 (1.1)	-0.39 (1.14)	0.826 (-0.35, 0.44)
Head circumference/Age z score Mean (SD)	0.49 (1.07)	0.51 (1.02)	0.941 (-0.42, 0.39)
BMI/Age z score Mean (SD)	0.77 (1.23)	1.17 (1.17)	0.090 (-0.86, 0.06)
Weight/Length z score Mean (SD)	0.79 (1.21)	1.18 (1.14)	0.084 (-0.85, 0.05)
MUAC/Age z score Mean (SD)	0.49 (1.22)	0.79 (1.05)	0.178 (-0.74, 0.14)
TSF/Age z score Mean (SD)	1.31 (0.35,1.63)	1.32 (0.47, 1.75)	0.485
Truncated Denver development score Median(range)	3 (3-3)	3 (3-8)	0.295

3.6.5.2 Longitudinal analyses using generalised estimating equations

(table 10 d-p, appendix 12)

All the above parameters were analysed further using generalised estimating equations (so that even the subjects who had missed visits could be included) with adjustments for follow-up time; mother's BMI; CD4 count; and socio-economic conditions.

There was a significant effect of time for all parameters (except the head circumference/age z score); both groups increased in the measurements over time equally. In comparison to the formula feeding infants, the breastfed control group significantly gained more weight and increased in HC; MUAC; TSF; and BMI measurements; in the BMI/age, TSF/age, weight/age and weight/length z scores. The HC/age z score was not different between groups or over time. All the measurements except for TSF; HC/age; TSF/age; and weight/length z scores were significantly higher in the infants whose mothers had a higher BMI. There was however no association with mother's CD4 counts. The infants of the mothers who had reported poor sanitation had significantly lower weights, lengths and z scores for weight for age; there was a borderline significance with BMI and z score for length for age. A group time interaction was noted for MUAC/age z score; the breastfed control infants had a slower rate of change in the MUAC than the formula fed infants.

Figure14: Mean weight for age z scores in the breastfeeding control and formula feeding groups

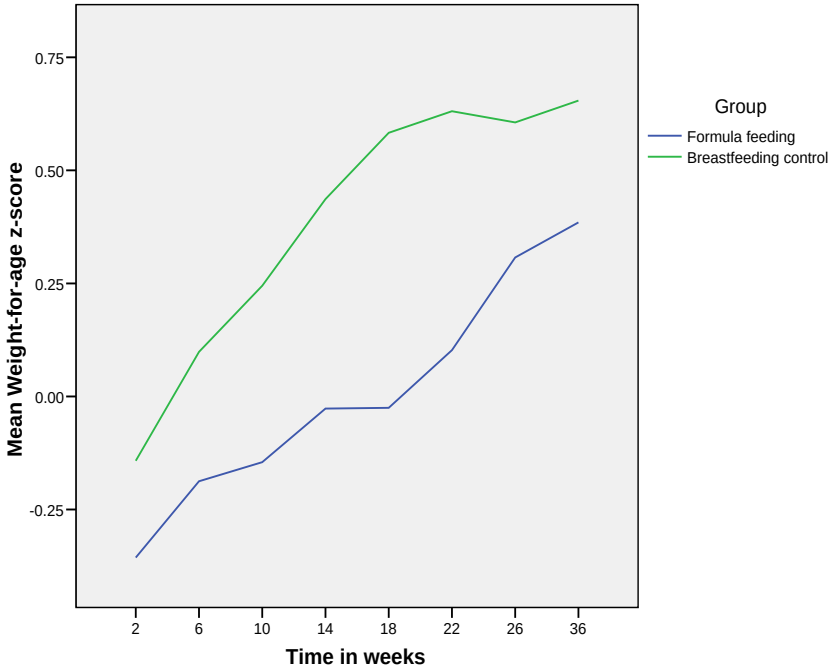


Figure15: Mean length for age z scores in the breastfeeding control and formula feeding groups

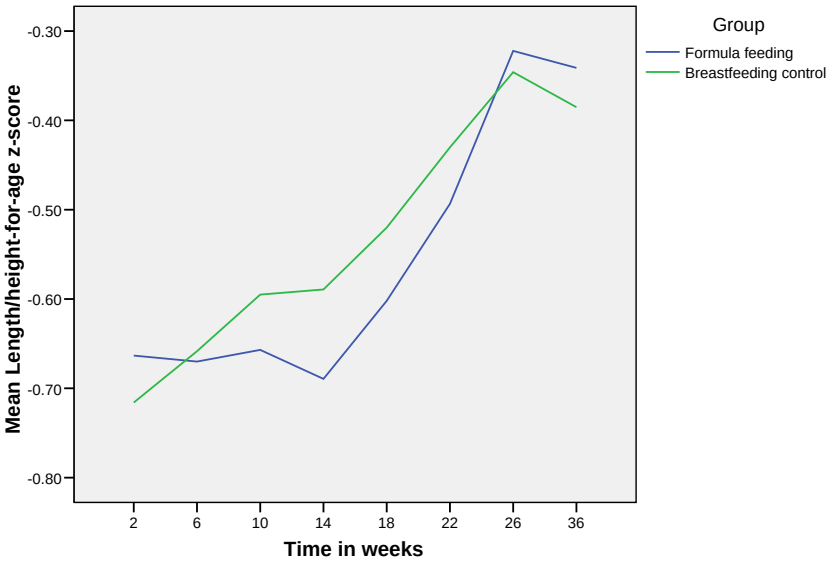


Figure16: Mean MUAC for age z scores in the breastfeeding control and formula feeding groups

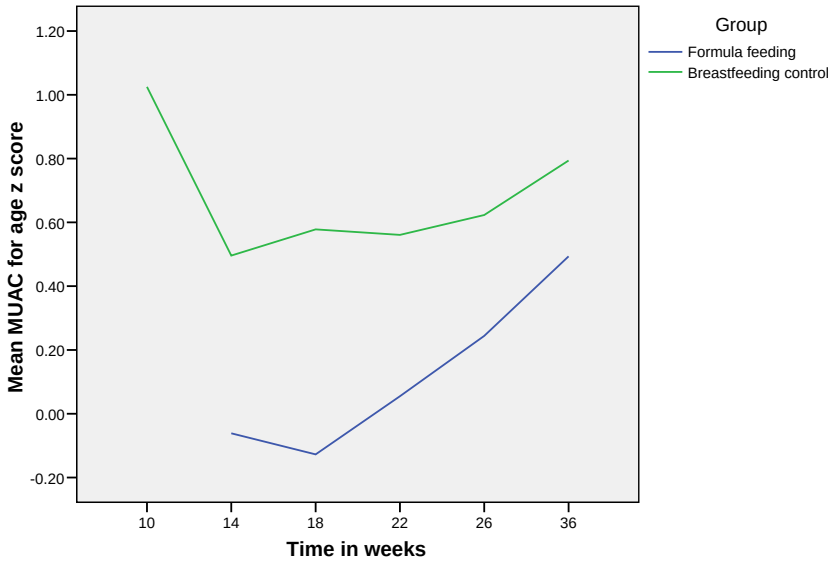
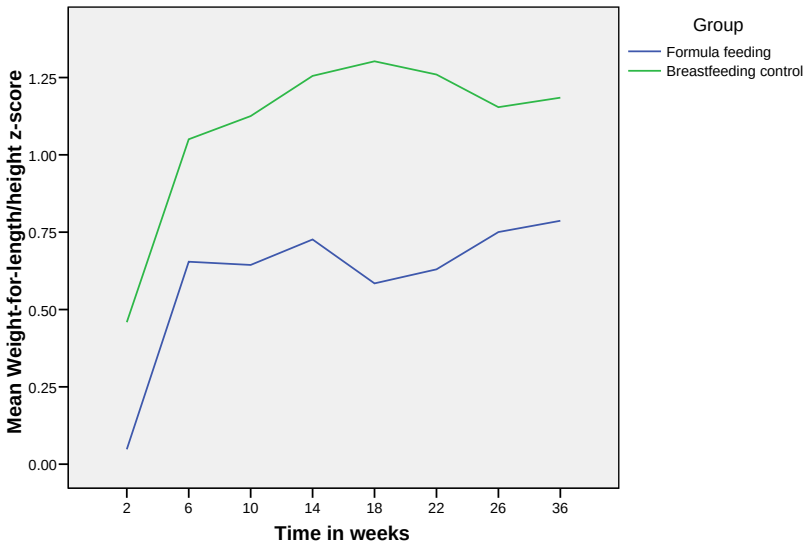


Figure17: Mean weight for length z scores in the breastfeeding control and formula feeding groups



3.6.6 Effect of breastfeeding on the incidence of opportunistic infections in the infant

3.6.6.1 Cross-sectional crude analyses at each time interval

2-14 weeks of age: There was a 69% lower incidence of diarrhoea in the breastfeeding control group compared to the formula feeding group (p=0.006); (see table 11a). There was also no incidence of hospital admissions in the breastfeeding group which was significantly different from the formula feeding group (p=0.014). There were no other significant differences noted in the incidence of any of the other opportunistic infections.

14 weeks to 6 months of age: There were no significant differences between the two groups; (see table 11b). Although there were more diarrhoeal events in the formula fed group, this difference was no longer significant.

6 months to 9 months of age: There were no significant differences between the two groups for incidence of any of the opportunistic infections; (see table 11c).

Table 11a: Effect of breastfeeding on incidence of opportunistic infections in the infant: crude incidence rates and incidence rate ratios per 1000 child week of follow-up between 2 and 14 weeks

Clinical conditions/events	Formula feeding (n=75)		Breastfeeding Control (n=63)		Incidence rate ratio (95% CI)	p value
	Count	Incidence rate	Count	Incidence rate		
Diarrhoea	23	21.99	6	6.8	0.31 (0.13-0.72)	0.006
Ear discharge	4	3.82	1	1.13	0.30 (0.04-2.33)	0.292
LRTI	2	1.91	0	0	n/a	0.294
FTT	1	0.96	0	0	n/a	0.543
TB	0	0	0	0	n/a	n/a
Skin sepsis	6	5.74	7	7.94	1.38 (0.47-4.1)	0.571
Meningitis	1	0.96	0	0	n/a	0.543
Delay in milestones	5	4.78	0	0	n/a	0.047
Admissions	7	6.69	0	0	n/a	0.014
Death	0	0	0	0	n/a	n/a

Table 11b: Effect of breastfeeding on incidence of opportunistic infections in the infant: crude incidence rates and incidence rate ratios per 1000 child week of follow-up between 14 weeks to 6 months of age

Clinical conditions/events	Formula feeding (n=75)		Breastfeeding Control (n=63)		Incidence rate ratio (95% CI)	P value
	Count	Incidence rate	Count	Incidence rate		
Diarrhoea	24	26.55	14	18.52	0.7 (0.36-1.34)	0.287
Ear discharge	2	2.21	4	5.29	2.39 (0.46-12.39)	0.337
LRTI	0	0	2	2.64	n/a	0.207
FTT	0	0	2	2.64	n/a	0.207
TB	3	3.32	5	6.61	1.99 (0.49-8.11)	0.365
Skin sepsis	3	3.32	1	1.32	0.4 (0.04-3.54)	0.470
Meningitis	0	0	0	0	0	n/a
Delay in milestones	4	4.42	4	5.29	1.2 (0.3-4.77)	0.807
Admissions	2	2.21	2	2.65	1.2 (0.17-8.47)	0.867
Death	0	0	0	0	0	n/a

Table 11c: Effect of breastfeeding on incidence of opportunistic infections in the infant: crude incidence rates and incidence rate ratios per 1000 child week of follow-up between 6 and 9 months of age

Clinical conditions/events	Formula feeding (n=75)		Breastfeeding Control (n=61)		Incidence rate ratio (95% CI)	p value
	Count	Incidence rate	Count	Incidence rate		
Diarrhoea	10	13.33	13	28.38	2.13 (0.95-4.76)	0.076
Ear discharge	1	1.33	0	0	0	0.621
LRTI	0	0	2	4.37	n/a	0.143
FTT	0	0	0	0	n/a	n/a
TB	0	0	2	4.37	n/a	0.143
Skin sepsis	0	0	1	2.18	n/a	0.379
Meningitis	0	0	1	2.18	n/a	0.379
Delay in milestones	0	0	2	4.37	n/a	0.143
Admissions	0	0	1	2.18	n/a	0.379
Death	0	0	2	4.37	n/a	0.143

3.6.6.2 Longitudinal analyses using Poisson distribution analyses in a generalized linear model (GLM) (table 11d-11m, appendix 13)

Generalised linear models with Poisson distribution and log function were used to analyse the data further. Diarrhoeal events which are an important indicator of child health in the 1st 3 months were assessed using GLM from 2 weeks to 3 months as well as longitudinally at end of follow-up. The breastfed group had significantly lower diarrhoeal events by 3 months (IRR=0.31; 95% CI 0.13-0.75). By end of follow-up, there was still a trend of higher diarrhoeal events in the formula fed infants, however the difference was no longer significant

(p=0.098). There were no significant differences between the two groups for the incidence of the other parameters.

3.6.7 Effect of breastfeeding on the growth and development in the infected infants

3.6.7.1 Cross-sectional analyses of growth

There were no significant differences between the two groups in terms of growth or development; (see table 12a). All groups had weight for age and height for age z scores lower than the reference range; Two infants at 14 weeks and three at six months were in the underweight category; two infants were stunted at 14 weeks and three at six months; two infants had wasting at 14 weeks and one at six months; all infants had normal weight for length z scores (see figures 18-21).

Table 12a: Growth and development in the infected children in the breastfeeding control and formula feeding

Growth parameters		**Formula feeding (n=3)**	**Breastfeeding control (n=5)**	**P value for the difference between the groups (95% CI)**
Weight/Age z score Mean (SD)	**2 week**	-0.22 (0.17)	-0.52 (0.9)	0.6 (-1.03, 1.63)
	14 week	-1.41 (1.4)	-0.31 (1.54)	0.352 (-3.78, 1.58)
	6 month	-0.91 (0.71)	-1.07 (1.57)	0.876 (-2.24, 2.57)
Length/Age z score Mean (SD)	**2 week**	-0.51 (0.51)	-1.28 (1.05)	0.292 (-0.86, 2.39)
	14 week	-1.92 (1.77)	-1.09 (-0.97)	0.412 (-3.14, 1.48)
	6 month	-1.48 (2.19)	-1.5 (0.78)	0.986 (-2.51, 2.54)
H C /Age z score Mean(SD)	**2 week**	0.52 (1.17)	0.36 (0.98)	0.851 (-1.93, 2.25)
	14 week	-0.49 (1.31)	0.42 (1.12)	0.335 (-3.03, 1.21)
	6 month	-0.42 (1.55)	-0.25 (1.77)	0.894 (-3.21, 2.87)
BMI/ Age z score Mean (SD)	**2 week**	0.09 (0.66)	0.23 (0.85)	0.816 (-1.56, 1.28)
	14 week	-0.41 (0.54)	0.43 (1.53)	0.406 (-3.13, 1.46)
	6 month	-0.02 (0.97)	-0.27 (1.73)	0.829 (-2.46, 2.96)
Weight/Length z score Mean (SD)	**2 week**	0.08 (0.91)	0.55 (0.81)	0.481 (-1.97, 1.04)
	14 week	0.36 (0.36)	0.78 (1.31)	0.622 (-2.36, 1.53)
	6 month	0.23 (1.19)	-0.09 (1.67)	0.778 (-2.4, 3.06)
MUAC for age z score Mean (SD)	**14 week**	-1.75 (1.75)	-0.02 (1.15)	0.137 (-4.19, 0.74)
	6 month	-0.98 (0.99)	-1.2 (1.35)	0.818 (-2, 2.44)
TSF for age z score Mean (SD)	**14 week**	-0.67 (2.09)	0.28 (0.59)	0.354 (-3.27, 1.37)
	6 month	0.29 (1)	-0.02 (1.04)	0.699 (-1.53, 2.14)
Truncated Denver developmental score	**14 week**	4 (4-6)	4 (4-4)	0.629
	6 month	3 (3-7)	3 (3-8)	1.000

Figure 18: Mean weight for age z scores over time in the infected infants

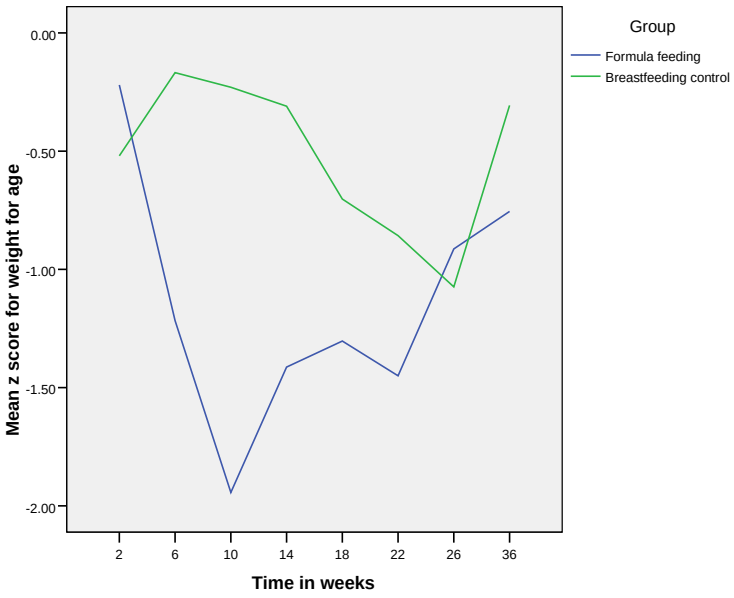


Figure 19: Mean length for age z scores over time in the infected infants

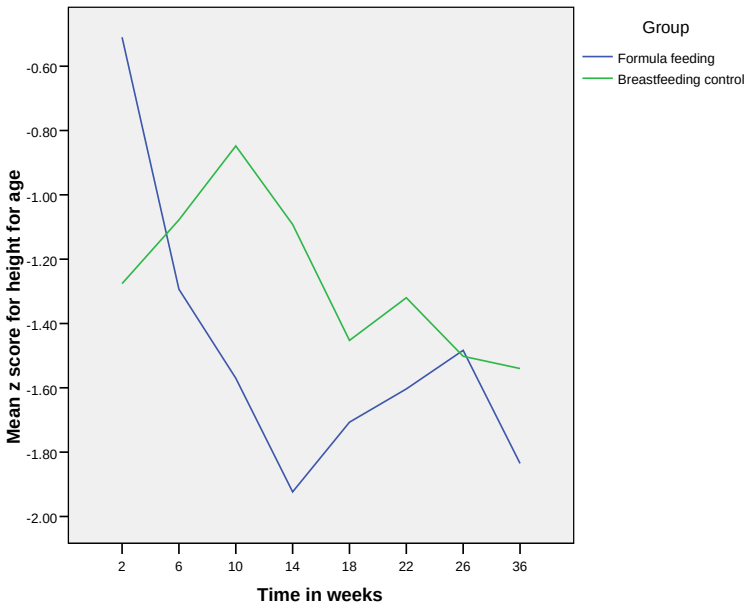


Figure 20: Mean weight for length z scores over time in the infected infants

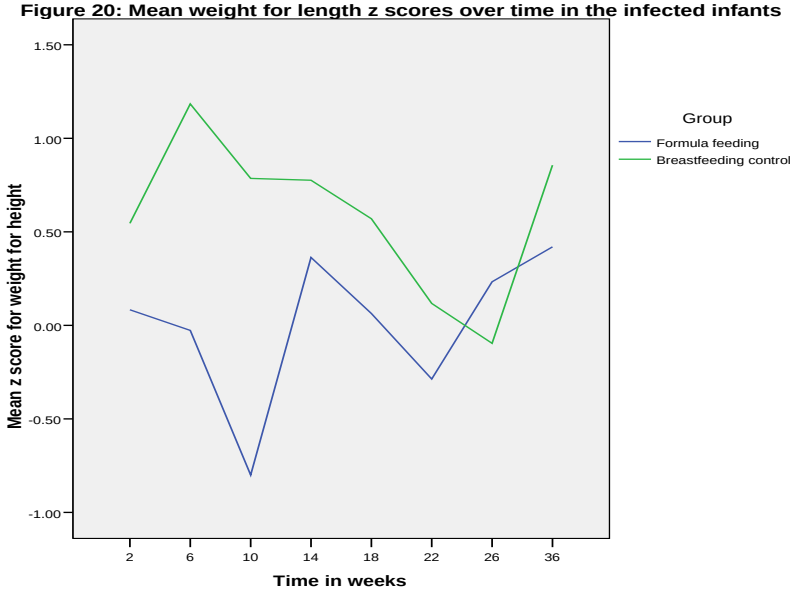
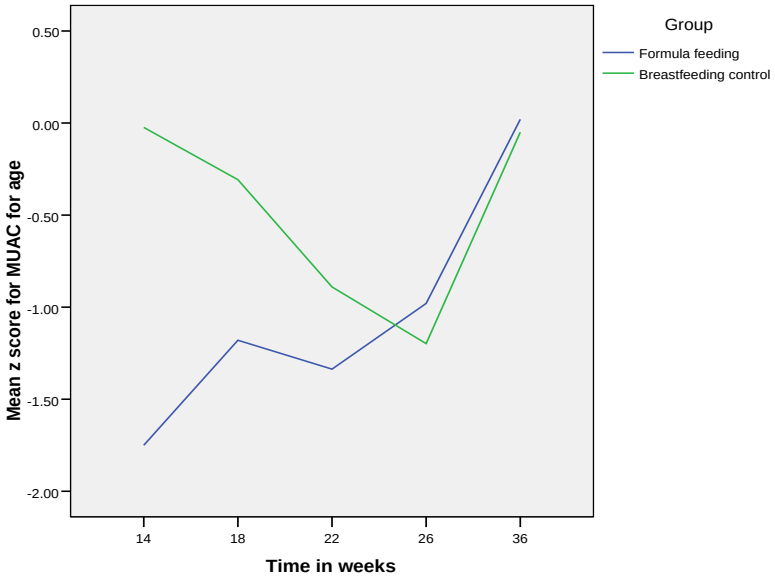


Figure 21: Mean MUAC for age z scores over time in the infected infants



3.6.7.2 Longitudinal analysis of growth over time using generalized estimating equations to study the effect of breastfeeding (table 12b-12n, appendix 14).

Generalised estimating equations with robust standard errors in an exchangeable covariance matrix were used to analyse the longitudinal data. There was a significant increase in all measurements over time except for the z scores for head circumference/age; weight/height and BMI/age i.e. infants in both groups gained equally over time. There was no effect of group or group time interaction. The breast fed infants had significantly higher TSF measurements and better z scores for MUAC/age and TSF/age. Infants of mothers with higher BMI; had higher length and TSF measurements and a higher z score for TSF/age. The infants with better socio-economic conditions in terms of electricity; water and sanitation also had higher length, TSF measurements and a higher TSF/age and MUAC/age z scores.

3.6.8 Effect of breastfeeding on disease progression amongst infants with HIV infection per 1000 child week of follow-up

3.6.8.1 Cross-sectional analyses over each time period

2 weeks to 3months: There were no significant differences amongst the vertically infected infants in both the groups for the incidence of any of the opportunistic infections; (table 13a). There was a significantly higher number of infants with developmental delay in the formula fed group (p=0.007) compared to the breastfed control group.

3 months to 6 months: There were no significant differences between the two groups; (table 13b). Although a trend could be seen of more visits of recording developmental delay amongst the formula fed infants; the difference did not reach significance.

6 months to 9 months: There was only one infected child who continued to breastfeed up to 9 months and there were no significant differences between the two groups.

Table 13a: Effect of breastfeeding on disease progression amongst infants with HIV infection per 1000 child week of follow-up between 2 and 14 weeks

Clinical conditions/events	Formula feeding (n=3)		Breastfeeding control (n=5)		Incidence rate ratio (95% CI)	p value
	Count	Incidence rate	Count	Incidence rate		
Oral thrush	1	23.81	2	28.57	1.2 (0.11-13.19)	0.928
Diarrhoea	2	47.62	1	14.29	2.26 (0.53-9.62)	0.272
Ear discharge	0	0	1	14.29	n/a	0.625
LRTI	1	23.81	0	0	n/a	0.375
FTT	0	0	0	0	n/a	n/a
TB	0	0	0	0	n/a	n/a
Skin sepsis	0	0	0	0	n/a	n/a
Meningitis	1	23.81	0	0	n/a	0.375
Delay in milestones	5	11.9	0	0	n/a	0.007
Admissions	2	47.62	0	0	n/a	0.141
Death	0	0	0	0	n/a	n/a

Table 13b: Effect of breastfeeding on disease progression amongst infants with HIV infection per 1000 child week of follow-up between 14 weeks to 6 months of age

Clinical conditions/events	Formula feeding (n=3)		Breastfeeding Control (n=5)		Incidence rate ratio (95% CI)	p value
	Count	Incidence rate	Count	Incidence rate		
Oral thrush	0	0	2	33.3	n/a	0.391
Diarrhoea	1	27.78	2	33.3	1.2 (0.11-13.19)	0.928
Ear discharge	1	27.78	1	16.67	0.6 (0.04-9.31)	0.75
LRTI	0	0	2	33.3	n/a	0.391
FTT	0	0	2	33.3	n/a	0.391
TB	1	27.78	4	66.67	2.4 (0.29-20.05)	0.477
Skin sepsis	0	0	0	0	n/a	n/a
Meningitis	0	0	0	0	n/a	n/a
Delay in milestones	4	11.11	1	16.67	0.15 (0.02-1)	0.077
Admissions	0	0	1	16.67	n/a	0.625
Death	0	0	0	0	n/a	n/a

3.6.8.2 Longitudinal analyses

Longitudinal analysis using generalised linear model with Poisson distribution and log function was done. No model could be formed for the incidence of skin sepsis as the models did not converge. No significant differences between the groups were seen over time for oral

thrush, diarrhoea, LRTI, ear discharge, TB, FTT, meningitis, admissions and death. The incidence of delayed milestones was significantly lower in the breastfed infants; (table 13c – 13l, appendix 15).

3.7 Effect of nutritional supplement on maternal and infant health

3.7.1 Adherence to nutritional supplement

Mothers in the supplemented group were issued the supplement for a median of 5.5 months; but they took the supplement appropriately for a median of 3.5 months (IQR 1.75-6); (see table 14 a-b). Assessed for associations between taking the supplement at an adequate dose with mothers' subjective likes and dislikes of taste revealed that there was a significant association between mothers' subjective feeling that the paste was not helping with reduced median intake (1 month) of the paste (p=0.002) and for feeling paste was too rich or sweet, with reduced median intake (2.5 months) of the paste (p=0.001). There was also a significant association between having no particular dislike of the paste and increased median months of intake (4.5 months) of the paste (p=0.01).

Table 14a: Intake of nutritional supplement

Indices	**Median(IQR)**
No. of months of breastfeeding	5.5(2.94-6)
No. of months supplement issued	5.5(3-6)
No. of months taken as advised	3.5(1.75-6)

Table 14b: Association of intake with subjective likes and dislikes of nutritional supplement

Subjective likes/dislikes of paste (n=66)		No (%)	Intake in months Median (min-max)	Mann-Whitney U test (p value)
What they liked about the paste	Increased energy	22 (33)	4.5 (1-6)	0.096
	Increased appetite and weight	27 (40.9)	4 (0-6)	0.487
	Increased Breastmilk	2 (3)	5 (4-6)	0.354
	Neutral or felt no particular advantages	15 (22.7)	1 (0-6)	0.002
What they disliked about the paste	Taste too rich and sweet	26 (39.4)	2.5 (0-6)	0.001
	Caused constipation	4 (6.1)	5.5 (4-6)	0.119
	No dislikes	36 (54.5)	4.5 (1-6)	0.01

3.7.2 *Analysis of the 24 hour dietary recall (table 15)*

There were no significant differences between the dietary intakes in both the breastfeeding groups for the components examined. The energy intake was within the RDA at baseline (≥ 2000 kcal≈ 8368Kj/day); although it did go below 2000 kcal/day at six and nine months. The total protein intake was within the lower normal RDA (46-50g/day) for a normal adult however it was below the RDA for lactating women (71g/day). Although the intake of the iron was within the RDA for a lactating mother; the haem iron component was very low. The vitamin A; folate and B12 intake were below the RDA for lactating mothers. Zinc intake was within the lower limits of the RDA.

Table 15: Comparison of dietary intake amongst the breastfeeding groups

Dietary component		Breastfeeding control (n=60)	Breastfeeding supplemented (n=58)	p value (95% CI)
Energy KJ Mean (SD)	**14 weeks**	8708.28 (3244.5)	8217.78 (2515.1)	0.362 (-570.5-1551.46)
	6 month	7031.75 (3656.1)	7481.43 (2614.6)	0.448 (-1618.85, 719.49)
	9 month	7373.97 (2552.9)	7827.22 (2552.3)	0.359 (-1427.87, 521.35)
Total protein g Median (IQR)	**14 weeks**	51.71 (38.69-71.7)	53.37 (41.58-69.24)	0.640
	6 month	42.8 (29.04-63.27)	50.9 (39.87-67.26)	0.037
	9 month	45.55 (35.52-61.15)	48.93 (38.77-69.25)	0.396
Plant protein g Median (IQR)	**14 weeks**	12.65 (8.47-19.66)	14.2 (9.6-21.42)	0.508
	6 month	12.66 (6.16-17)	13.02 (8.27-17.73)	0.497
	9months	12.73 (8.09-18.66)	14.54 (8.94-22.07)	0.299
Animal protein g Median (IQR)	**14 weeks**	18.49 (8.09-36.36)	18 (6.87-32.29)	0.848
	6 month	16.66 (4.79-24.7)	21.61 (7.48-32.81)	0.174
	9 month	16.14 (8.54-28.2)	18.18 (7.57-25.86)	0.983
Total fat g Mean (SD)	**14 weeks**	62.84 (39.6)	60.23 (33.9)	0.702 (-10.86, 16.07)
	6 months	50.43 (37)	53.32 (25.2)	0.625 (-14.54, 8.77)
	9 months	54.52 (28.6)	57.97 (29.1)	0.535 (-14.47, 7.56)
Saturated FA g Median (IQR)	**14 weeks**	15.14 (7.15-23.69)	15.24 (8.34-22.32)	0.844
	6 months	11.33 (5.87-20.09)	12.98 (8.43-18.73)	0.297
	9 months	13.7 (7.8-17.4)	12.1 (9.11-18.5)	0.919
Monounsaturated FA g Median (IQR)	**14 weeks**	17.44 (9.62-22.25)	15.7 (9.67-25.7)	0.897
	6 months	12.62 (7.8-19.52)	14.06 (11-20.68)	0.191
	9 months	14.87 (10.7-22.1)	15.06 (10-23.37)	0.820
Polyunsaturated FA g Mean (SD)	**14 weeks**	19.06(14.3)	18.37(13.2)	0.787 (-4.34, 5.72)
	6 months	15.44(12.5)	15.41 (8.58)	0.990 (-3.93, 3.98)
	9 months	15.38 (9.2)	18.37 (10.28)	0.116 (-6.72, 0.75)
Carbohydrate g Mean (SD)	**14 weeks**	306.42(106.41)	280.94(97.94)	0.179 (-11.84, 62.8)
	6 months	245.54 (122.47)	257.75 (96)	0.551 (-52.69, 28.27)
	9 months	252.13 (84.99)	266.53 (90.85)	0.398 (-48.03, 19.23)
Starch g Mean (SD)	**14 weeks**	123.42 (65.16)	123.45 (71.16)	0.998 (-24.89, 24.84)
	6 months	94.22 (72.83)	109.96 (66.31)	0.226 (-41.36, 9.88)
	9 months	103.75 (52.11)	105.46 (68.1)	0.885 (-24.97, 21.56)
Fe mg Mean (SD)	**14 weeks**	15.14 (5.9)	14.9 (5.17)	0.829 (-1.81, 2.25)
	6 months	11.85 (6.92)	14.92 (5.18)	0.072 (-4.72, 0.2)
	9 months	13.58 (5.22)	14.16 (5.33)	0.571 (-2.59, 1.43)
Haem iron mg Mean (SD)	**14 weeks**	0.13 (0-0.52)	0.03 (0-0.32)	0.330
	6 months	0.13 (0-0.3)	0.13 (0-0.47)	0.722
	9 months	0.09 (0-0.34)	0.12 (0-0.38)	0.912
Nonhaem iron Mean (SD)	**14 weeks**	2.28 (1.47-4.1)	2.37 (1.48-3.37)	0.583
	6 months	2.24 (1.18-3.02)	2.77 (1.64-3.98)	0.119
	9 months	1.96 (1.18-3.44)	2.25 (1.4-4.09)	0.167
Zinc mg Mean (SD)	**14 weeks**	11.71 (6.02)	11.38 (4.3)	0.731 (-1.58, 2.25)
	6 months	9.26 (5.7)	10.53 (4.86)	0.197 (-3.22, 0.67)
	9 months	10.24 (4.23)	10.68 (4.61)	0.601 (-2.14, 1.24)
Retinol µg Median (IQR)	**14 weeks**	9.4 (0-73.4)	4.08 (0-64.58)	0.360
	6 months	7.6 (0-34.25)	28.35 (0-58.64)	0.257
	9 months	5.2 (0-46.54)	19.2 (0-63.2)	0.387
Folate µg Median (IQR)	**14 weeks**	309.7 (235.6-472.8)	321.31 (242-471.3)	0.750
	6 months	263.6 (114.7-453.3)	324.48 (197.8-460.4)	0.183
	9 months	298.9 (211.6-425.3)	327.8 (243.04-435.6)	0.698
B12 µg Mean (SD)	**14 weeks**	1.26 (0.25-4.14)	1.69 (0.3-2.9)	0.893
	6 months	0.97 (0.2-2)	1.31 (0.54-2.6)	0.074
	9 months	1.31 (0.5-2.57)	1.67 (0.47-3.19)	0.593

3.7.3 Effect of nutritional supplementation on body composition and anthropometric measures of breastfeeding mothers

3.7.3.1 Cross-sectional analysis at 14 weeks

There were no significant differences between the two breastfeeding groups at 14 weeks post delivery (Table 16a).

Table 16a: Effect of nutritional supplementation on Body composition and anthropometric measures of breastfeeding mothers at 14 weeks

Parameter Mean (SD)	Breastfeeding Control (n=60)	Breastfeeding Supplemented (n=57)	P value for the difference between the groups (95% CI)
Weight in kg	65.64 (10.07)	64.97 (10.5)	0.728 (-3.1, 4.43)
BMI in kg/m^2	26.7 (4.19)	26.4 (4.6)	0.712 (-1.31, 1.91)
MUAC in cm	28.58 (3.12)	28.68 (3.57)	0.861 (-1.33, 1.12)
TSF in mm	19.53 (4.82)	19.22 (6.03)	0.756 (-1.68, 2.31)
LBM in kg	42.52 (5.2)	41.57 (4.95)	0.394 (-1.26, 3.15)
Fat mass in kg	22.63 (7.53)	22.41 (6.88)	0.888 (-2.91, 3,36)
%Fat mass	34.08 (7.21)	34.44 (7)	0.817 (-3.45, 2.73)

3.7.3.2 Cross-sectional analysis at 6 months (table 16b)

There was a drop in weight, BMI and lean body mass in both the supplemented and non-supplemented mothers; the drop was similar in the two groups. The MUAC, TSF; fat mass and percentage fat mass all increased in both groups of mothers and this increase was not significantly different between the two groups.

Table 16b: Effect of nutritional supplementation on Body composition and anthropometric measures of breastfeeding mothers from 2 weeks to 6 months

Mean change in variable over 6 months (sd)	Breastfeeding Control (n=57)	Breastfeeding Supplemented (n=56)	P value for the difference between the groups (95% CI)
Weight in kg	-0.43 (4.78)	-1.61 (4.89)	0.196 (-0.62, 2.97)
BMI in kg/m^2	-0.13 (1.94)	-0.64 (1.97)	0.164 (-0.21, 1.24)
MUAC in cm	1.20 (1.62)	-0.90 (1.73)	0.349 (-0.33, 0.92)
TSF in mm	4.14 (4.69)	2.87 (4.88)	0.161 (-0.51, 3.05)
LBM in kg	-3.78 (4.1)	-3.20 (3.52)	0.569 (-2.59, 1.44)
Fat mass in kg	4.14 (4.57)	3.21 (5.77)	0.490 (-1.76, 3.63)
%Fat mass	6.07 (5.46)	4.5 (6.53)	0.316-1.55, 4.70)

3.7.3.3 Sub-group analysis of the effect of nutritional supplementation on body composition and anthropometric measures of breastfeeding mothers by BMI category (table 16c)

A sub-group analysis i.e. studying the changes in the variables within BMI category ≤ 24.99 kg/m^2 and ≥ 25 kg/m^2 was carried out. The changes were not significantly different within the subgroup analysis except for the lean body mass. The LBM decreased in both groups of subjects with the low BMI, however there was a statistically significant lower mean decrease in the supplemented group (1.32 kg) when compared to the control group (3.17 kg); p=0.026. This was not reciprocated in the subjects with the high BMI as the mean decrease was similar in both groups.

The mean percentage fat mass increase was significantly higher in the control subjects (7.171%) with the low BMI when compared to the supplemented group (2.55%); p=0.031. In the subjects with the high BMI, the difference between the groups was however not statistically significant (p=0.643).

Table 16c: Sub-group analysis of the effect of nutritional supplementation on Body composition and anthropometric measures of breastfeeding mothers (by BMI category)

Change in variable over 6 months	Low to normal BMI (≤24.99 kg/m^2)			High BMI (≥ 25 kg/m^2)		
	Breastfeeding Control (n=22)	Breastfeeding Supplemented (n=27)	P value for the difference between the groups (95% CI)	Breastfeeding Control (n=35)	Breastfeeding Supplemented (n=30)	p value for the difference between the groups (95% CI)
Weight kg Mean (SD)	0.3 (4.45)	-0.94 (3.9)	0.306 (-1.17, 3.64)	-0.89 (4.99)	-2.22(5.63)	0.318 (-1.31,3.96)
BMI kg/m^2 Mean (SD)	0.23 (1.76)	-0.36 (1.51)	0.215 (-0.35, 1.53)	-0.36 (2.04)	-0.90 (2.31)	0.316 (-0.53, 1.62)
MUAC cm Mean (SD)	1.44(1.61)	0.69(1.35)	0.083 (-0.10, 1.60)	1.05 (1.64)	1.10 (2.03)	0.910 (- 0.97, 0.86)
TSF mm Mean (SD)	4.45(4.15)	3.11(4.53)	0.291 (-1.18, 3.86)	3.95(5.04)	2.65(5.26)	0.318 (-1.28, 3.88)
LBM kg Mean (SD)	-3.71 (2.76)	-1.32(2.46)	0.026 (-4.46,- 0.32)	-3.84(5.2)	-4.71(3.57)	0.597 (-2.44,4.16)
Fat mass kg Mean SD)	4.58 (3.86)	1.72(4.7)	0.090 (-0.47, 6.18)	3.71(5.28)	4.4 (6.4)	0.747 (-4.98, 3.62)
%Fat mass Mean (SD)	7.17(4.87)	2.55(5.82)	0.031 (0.46, 8.77)	4.98 (5.93)	6.05 (6.84)	0.643 (-5.77, 3.62)

3.7.3.4 Assessment of changes in anthropometric and body composition parameters over 6 months per category of duration of breastfeeding/supplementation (Table 16d)

As there was a variation in the duration of breastfeeding and therefore intake of the supplement, we assessed for intra group differences within the supplemented group. There were no significant differences for any of the anthropometric measures or body composition measures for any of the indices per duration of supplementation/breastfeeding.

Table 16d: Sub-group analysis: Effect of the duration of breastfeeding/supplementation on changes in all anthropometry and body composition measures over 6 months

Change in variable over 6 months	Duration of breastfeeding/supplementation		
	≤ 3.5 months(n=16)	**>3.5 months(n=42)**	**p value for the difference between the groups (95% CI)**
Weight in kg Mean (SD)	-2.4 (6.66)	-1.06 (4.8)	0.430 (-2.04, 4.71)
BMI in kg/m^2 Mean (SD)	-0.94 (2.71)	-0.43 (1.91)	0.460 (-0.85, 1.86)
MUAC in cm Mean (SD)	1.01 (2.24)	1.02 (1.7)	0.991 (-1.17, 1.18)
TSF in mm Mean (SD)	3.53 (5.72)	2.57 (4.37)	0.523 (-3.96, 2.04)
LBM in kg Mean (SD)	-4.56 (3.69)	-2.95 (3.62)	0.353 (-1.91, 5.13)
Fat mass in kg Mean (SD)	5.39 (8.11)	3.07 (4.88)	0.397 (-7.88, 3.24)
%Fat mass Mean (SD)	7.07 ((8.86)	4.28 (5.74)	0.371 (-9.13, 3.54)

3.7.3.5 Longitudinal analysis of body composition and anthropometric measures

Further analysis using generalised estimating equations adjusted for baseline age, CD4 and duration of breastfeeding was conducted in both groups. Data was analysed separately for mothers with BMI ≤ 24.99 kg/m^2 and ≥ 25 kg/m^2.

<u>BMI ≥ 25 kg/m^2 (table 16e-16k, appendix 16)</u>

Fat mass, percentage fat mass, MUAC, TSF increased over time equally in the two groups. LBM decreased equally in both groups. There was no effect of supplementation on any of the parameters. However duration of breastfeeding was independently associated with body composition changes. Women who breastfed for short periods (≤ 3.5 months) significantly

lost more weight (3.96kg); had lower fat mass (4.42 kg); lower percentage fat mass (3.72); and lower TSF measurements (2.93mm) than the mothers who breastfed for longer.

BMI ≤ 24.99kg/m^2 (tables 16l-16r, appendix16)

Fat mass, MUAC and TSF measurements increased with time equally in the two breastfeeding groups. Older mothers had higher weight, BMI and MUAC regardless of time and group. The supplemented group had a significantly lower loss in LBM (0.098kg) compared to the control group. Therefore even when controlling for baseline age, CD4 and duration of breastfeeding, supplementation had a positive effect on LBM.

Unlike the group of mothers with a high BMI, in this group of mothers with a low BMI breastfeeding duration was not associated with any significant changes in body composition.

3.7.4 Effect of nutritional supplement on the laboratory parameters of breastfeeding mothers at 6 months

3.7.4.1 Effect of the nutritional supplement on the laboratory parameters

There were no significant differences between the changes for all the parameters in both groups; (see table 17a).

Table 17a: Effect of nutritional supplementation on laboratory parameters of breastfeeding mothers

Change in variable over 6 months	Breastfeeding control(n=52)	Breastfeeding supplemented(n=52)	p value for the difference between the groups (95% CI)
Haemoglobin g/dL Mean (SD)	0.33 (1.41)	0.13 (1.35)	0.460 (-0.33,0.73)
Haematocrit % Mean (SD)	1.04 (3.5)	0.41 (3.67)	0.371 (-0.77, 2.03)
MCV fl Mean (SD)	-5.89 (5.7)	6.63 (6.8)	0.552 (-1.71, 3.19)
Platelet count x 10^9/L Mean (SD)	-100.75 (96.76)	-109.82 (124.42)	0.679 (-34.24, 52.38)
Total lymphocyte count x 10^9/L Mean (SD)	0.1 (0.70)	0.002 (0.59)	0.464 (-0.17, 0.36)
Total Protein g/L Mean (SD)	8.16 (8.36)	7.04 (7.23)	0.47 (-1.96, 4.20)
Albumin g/L Mean (SD)	3.96 (5.26)	4.89 (3.42)	0.293 (-2.66, 0.81)
White cell count x 10^9/L Median(IQR)	-0.40 (-1.7, 0.4)	-0.50 (-1.58, 0.53)	0.847
CD4 cells/µL Median(IQR)	8 (-89.5, 73.5)	-9 (-76.75, 76.25)	0.660

3.7.4.2 Sub-group analysis of the effect of nutritional supplementation on the laboratory parameters of breastfeeding mothers at 26 weeks

A sub-group analysis i.e. studying the changes in the variables within BMI category ≤ 24.99 kg/m^2 and ≥ 25 kg/m^2 was carried out; (see table 17b).

The difference between the groups was not statistically significant for the changes in any of the blood parameters.

Table 17b: Sub-group analysis of the effect of nutritional supplementation on laboratory parameters of breastfeeding mothers (by BMI category)

Change in variable over 6 months	Low to normal BMI (≤24.99 kg/m^2)			High BMI (≥ 25 kg/m^2)		
	Breastfeeding Control (n=18)	**Breastfeeding Supplemented (n=24)**	**p value for the difference between the groups (95% CI)**	**Breastfeeding Control (n=33)**	**Breastfeeding Supplemented (n=30)**	**p value for the difference between the groups (95% CI)**
Haemoglobin g/dL Mean (SD)	0.48 (1.24)	-0.15 (1.41)	0.132 (-0.2, 1.45)	0.25 (1.51)	0.37 (1.28)	0.734 (-0.83, 0.59)
Haematocrit % Mean (SD)	0.49 (3.26)	-0.39 (3.56)	0.416 (-1.28,3.04)	1.36 (3.64)	1.05 (3.7)	0.746 (-1.56,2.17)
MCV fl Mean (SD)	-5.6 (6.75)	-5.02 (7.14)	0.791 (-4.98, 3.82)	-6.05 (5.12)	-7.91 (6.34)	0.207 (-1.06,4.78)
Platelet count x 10^9/L Mean (SD)	-87.83 (69.45)	-108.8 (130.8)	0.540 (-47.7, 89.7)	-107.79 (109.18)	110.6 (121.28)	0.923 (-60.87, 55.25)
Lymphocyte x 10^9/L Mean (SD)	-0.07 (0.56)	0.15 (0.53)	0.231 (-0.57, 0.14)	0.18 (0.76)	-0.14 (0.62)	0.096 (-0.59, 0.67)
Total Protein g/L Mean (SD)	9.53 (1.96)	5.64 (6.48)	0.103 (-0.83, 8.62)	7.44 (8.53)	8.07 (7.68)	0.762 (-4.76,3.5)
Albumin g/L Mean (SD)	4.06 (3.87)	4.23 (3.52)	0.884 (-2.54, 2.2)	3.91 (5.96)	5.37 (3.32)	0.242 (-3.94, 1.01)
WCC x 10^9/L Median(IQR)	0.1 (-0.45, 0.45)	-0.1 (-0.8, 0.78)	0.533	0.90 (-2.3, 0.45)	-0.75 (-2.03, 0.23)	0.804
CD4 cells/µL Median(IQR)	27.5(-89.3, 99.3)	11 (-26, 39)	0.733	-43 (-92, 66)	7 (-97, 85.5)	0.513

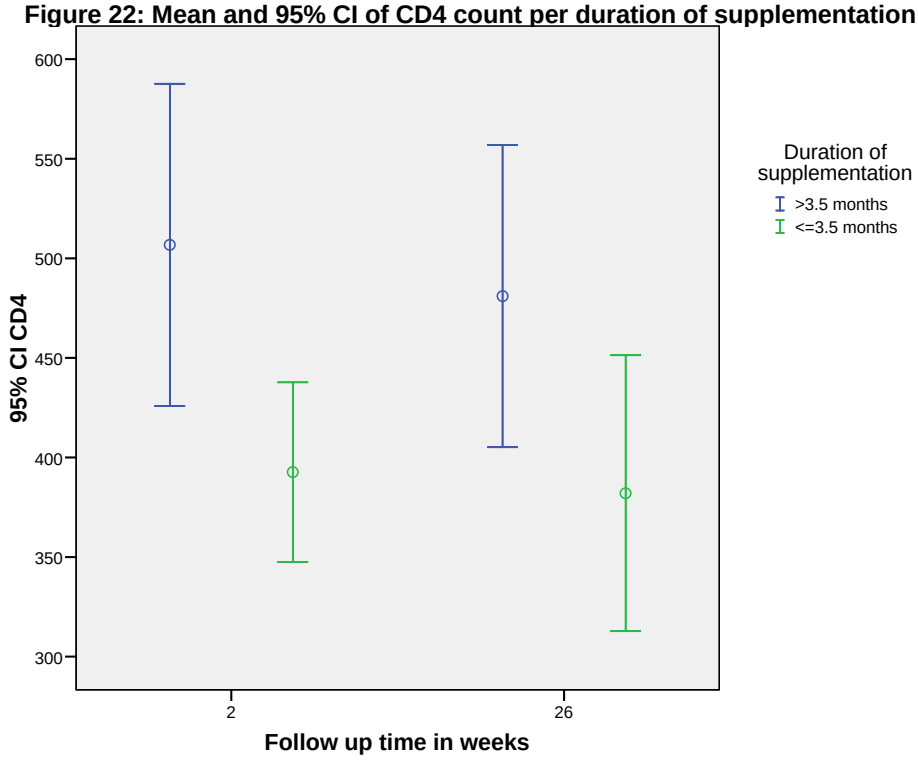
3.7.4.3 Haematological and Biochemical indices per duration of breastfeeding/ supplementation (table 17c)

The supplemented group was assessed further for any intra group differences as per duration of breastfeeding and hence supplementation. There were no significant differences between the laboratory parameters for any of the analyses except for CD4 count which increased by a median of 22 cells/µLin the group that breastfed for longer than 3.5 months compared to a decrease by 50 cells/ mm3 in the group who breastfed for 3.5 months or less (p=0.037).

Table 17c: Sub-group analysis: Effect of duration of supplementation on changes in all measured laboratory parameters over 6 months

Change in variable over 6 months		Issue of supplement		
		≤ 3.5 months(n=16)	> 3.5 months(n=42)	P value for the difference between the groups (95% CI)
Haemoglobin g/dL	**Mean (SD)**	0.36 (1.68)	0.27 (1.32)	0.842 (-0.99, 0.81)
Haematocrit %	**Mean (SD)**	1.0 (4.17)	0.58 (3.72)	0.913 (-2.30, 2.57)
MCV fl	**Mean (SD)**	-7.81 (7.81)	-6.38 (6.57)	0.513 (-2.95, 5.83)
Platelet count x 10^9/L	**Mean (SD)**	-129.21 (132.54)	-104.06 (107.39)	0.490 (-47.51, 97.83)
TLC x 10^9/L	**Mean (SD)**	-0.11 (0.49)	0.03 (0.66)	0.487 (-0.26, 0.53)
Total Protein g/L	**Mean (SD)**	7.92 (11.11)	7.03 (5.82)	0.721 (-5.88, 4.1)
Albumin g/L	**Mean (SD)**	5.5 (4.7)	4.78 (3.19)	0.551 (-3.14, 1.7)
WCC x 10^9/L	**Mean (SD)**	-1.43 (2.28)	-0.35 (1.74)	0.075 (-0.12, 2.29)
CD4 cells/µL	**Median(IQR)**	-50(-120, 38)	22 (-57, 83)	0.037

Figure 22: Mean and 95% CI of CD4 count per duration of supplementation



3.7.4.4 Longitudinal analyses of CD4 counts (table17d, appendix 17)

In order to investigate further whether duration of breastfeeding had an independent impact on the CD4 count, we conducted a longitudinal analysis in the two groups using

generalized estimating equations controlling for age and duration of breastfeeding. The effect of supplementation on CD4 was no longer evident and instead we observed a significantly higher mean CD4 count in the group with a longer duration of breastfeeding (74 cells; p=0.040).

3.7.5 Effect of nutritional supplement on selected micronutrients of breastfeeding mothers over 26 weeks

3.7.5.1 Effect of nutritional supplementation on selected micronutrients

There were no significant differences between the two breastfeeding groups for the changes in any of the micronutrients over time; (see table 18a). The mean increase in B12 levels was higher in the supplemented group (40.62 pg/ml) in comparison to the control group (34.17 pg/ml); however the difference was not significant (p=0.838).

Table 18a: Effect of nutritional supplement on selected micronutrients of breastfeeding mothers

Change in parameter over 6 months	Breastfeeding control(n=57)	Breastfeeding supplemented(n=56)	p value for the difference between the groups (95% CI)
Folate ng/mL Mean (SD)	-0.62 (4.57)	0.62 (6.24)	0.363 (-3.95, 1.46)
Zinc µg/mL Mean (SD)	4.99 (10.63)	4.44 (7.23)	0.808 (-3.96, 5.06)
B12 pg/mL Mean (SD)	34.17 (127.09)	40.62 (123.03)	0.838 (-69.33, 56.43)

3.7.5.2 Sub-group analysis of the effect of nutritional supplementation on selected micronutrients of breastfeeding mothers over 26 weeks by BMI category

There were similar changes in both groups, no significant differences between the groups were noted for any of the micronutrients; (see table 18b). The micronutrients in the serum

within the subgroup could not be analysed as well as they were done in a subsample of the entire cohort.

Table 18b: Sub-group analysis of the effect of nutritional supplementation on selected micronutrients of breastfeeding mothers (by BMI category)

Change in parameter over 6 months	Low to normal BMI (≤24.99 kg/m^2)			High BMI (≥ 25 kg/m^2)		
	Breastfeeding Control (n=13)	Breastfeeding Supplemented (n=12)	p value for the difference between the groups (95% CI)	Breastfeeding Control (n=23)	Breastfeeding Supplemented (n=18)	p value for the difference between the groups (95% CI)
Folate ng/ml Mean (SD)	-0.99 (4.70)	0.46 (6.06)	0.508 (-5.92,3.02)	-0.40 (4.58)	-0.73 (6.55)	0.529 (-4.75, 2.48)
Zinc µg/ml Mean (SD)	2.54 (11.56)	2.31 (7.75)	0.953 (-7.74, 8.2)	6.37 (10.07)	5.97 (6.62)	0.886 (-5.16, 5.96)
B12 pg/ml Mean (SD)	-4.85 (123.87)	28.75 (114.87)	0.49 (-132.68, 65.49)	57.23 (126.03)	49 (131.3)	0.844 (-75.74, 92.2)

3.7.6 Effect of nutritional supplement on disease progression, quality of life and depression scores of the breastfeeding mothers

3.7.6.1 Cross-sectional analysis at six months

There were no significant differences in the crude incidence rates or incidence rate ratios between the breastfeeding mothers receiving supplementation and the control group; (see table 19a).

Table 19a: Effect of nutritional supplement on disease progression, quality of life and depression scores: Crude incidence rates and incidence rate ratios of various opportunistic infections and other clinical events per 1000 person week of follow-up

Clinical events	Breastfeeding: control (n=63)		Breastfeeding : supplemented (n=66)		Incidence rate ratio (95% CI)	p value
	Events	Crude incidence rate	Events	Crude incidence rate		
Oral thrush	0	0	1	0.56	0	0.474
PID	8	4.45	7	3.51	0.79 (0.29-2.17)	0.657
LRTI	3	1.67	6	3.01	1.81 (0.46-7.08)	0.425
Admissions	1	0.56	0	0	0	0.474
Breast pathology	5	2.78	6	3.01	1.08 (0.33-3.55)	0.904
Genital tract infections	32	17.8	39	19.58	1.1 (0.69-1.76)	0.693
TB	0	0	1	0.56	0	0.474
Herpes simplex	1	0.56	3	1.51	2.71 (0.31-23.77)	0.428
SRQ score ≥ 8	6	3.34	12	6.02	1.80 (0.69-4.74)	0.242
Karnofsky score ≤ 80	1	0.56	6	3.01	5.42 (0.82-35.66)	0.092

3.7.6.2 Analysis for the effect of nutritional supplementation on disease progression, quality of life and depression scores per category of duration of supplement

On doing a subgroup analysis to assess the differences within the supplemented group for duration of supplementation; we observed significant differences between the group that took the supplement for 3.5 months or less compared to the group that took the supplement for greater than 3.5 months; (table 19b). The incidence rates were higher for the events of pelvic inflammatory disease, lower respiratory tract infections, breast pathology and a lower Karnofsky score amongst the mothers who took the supplement for 3.5 months or less.

Table 19b: **Sub-group analysis of the effect of the duration of supplementation on disease progression, quality of life and depression scores: incidence rates and rate ratios per 1000 person week of follow-up**

Clinical events	≤ 3. 5 months (n=16)		> 3.5 months (n=42)		Incidence rate ratio (95% CI)	p value
	Events	Crude incidence rate	Events	Crude incidence rate		
PID	5	9.8	2	1.35	7.26 (1.79-29.43)	0.016
LRTI	4	7.84	2	1.35	5.81 (1.3-25.96)	0.046
Breast pathology	4	7.84	2	1.35	5.81 (1.3-25.96)	0.046
Genital tract infections	14	27.45	25	16.87	1.63 (0.85-3.11)	0.154
TB infection	1	1.96	0	0	0	0.256
Herpes simplex	2	3.92	1	0.67	5.81 (0.7-48.25)	0.180
SRQ score ≥ 8	8	10.34	9	6.33	1.63(0.55-4.77)	0.322
Karnofsky score ≤ 80	6	11.76	0	0	0	<0.001

***3.7.6.3** Longitudinal analyses of the morbidity using generalized linear model with Poisson distribution (table 19c-19i, appendix 18)*

On adjusting for CD4, age and duration of breastfeeding, we found no significant differences between the groups for the incidence rates of all the opportunistic infections except for genital ulcers. The breastfeeding supplemented group had a significantly lower risk of genital ulcers as compared to the non-supplemented group.

Shorter duration of breastfeeding (≤ 3.5 months) was significantly associated with a higher

breast pathology (IRR 8.29; 95% CI 2.44, 28.2); risk of PID (IRR 5.75; 95% CI 1.93, 17.16); and genital ulcers (IRR 2.65; 95% CI 1.62, 4.35). Older mothers had a significantly lower risk of genital ulcer; breast pathology; and PID.

3.7.7 Effect of maternal nutritional supplementation on growth and development of the breastfed infant

3.7.7.1 Cross-sectional analyses

Growth and development at 14 weeks: The MUAC, TSF, BMI measurements as well as the z scores for weight for age, weight for length, MUAC and TSF were significantly higher in the breastfed control group compared to the supplemented group; (table 20 a). None of the measurements were below one standard deviation of the reference. There was no significant difference between the mean modified Denver development score for either group**.**

Growth and development at 6 months: The weight, TSF, BMI measurements as well as the z scores for weight for age, weight for length, BMI and TSF was significantly higher in the breastfed control group compared to the supplemented group; (see table 20b). None of the measurements were below one standard deviation of the reference. There were no significant differences between the mean modified Denver development scores in either group.

Growth and development at 9 months: The TSF measurement and the z scores for BMI, weight for length and TSF for age were significantly higher in the breastfed control group; (table 20c). No z scores were more than one standard deviation below the reference. There were no significant differences between the mean Denver development scores in either of the two groups.

Table 20a: Effect of maternal supplementation with micronutrient enriched paste on growth and development of the breastfed infant at 14 weeks

Growth and development parameters	Breastfeeding control (n=63)	Breastfeeding supplemented (n=66)	p value for the difference between the groups
Weight kg Mean (SD)	6.73 (0.89)	6.43 (0.83)	0.066 (-0.02, 0.61)
Length cm Mean (SD)	60.28 (2.62)	60.49 (2.51)	0.667 (-1.15, 0.74)
Head circumference cm Mean (SD)	41.04 (1.51)	41.32 (1.44)	0.304 (-0.82, 0.26)
MUAC cm Mean (SD)	13.95 (1.09)	13.47 (1.08)	0.017 (0.09, 0.89)
TSF mm Mean (SD)	10.93 (1.46)	9.96 (1.67)	0.001 (0.4, 1.55)
BMI kg/m^2 Mean (SD)	18.45 (1.59)	17.54 (1.66)	0.003 (0.31, 1.51)
Weight z scores Mean (SD)	0.44 (1.03)	0.06 (1.09)	0.058 (-0.01, 0.77)
Length for age z score Mean (SD)	-0.59 (1.08)	-0.48 (1.12)	0.613 (-0.51, 0.3)
Head circumference for age z score Mean (SD)	0.50 (1.07)	0.73 (1.1)	0.249 (-0.63, 0.17)
BMI for age z score Mean (SD)	1.06 (0.98)	0.46 (1.11)	0.058 (-0.01, 0.77)
Weight for length z score Mean (SD)	1.26 (0.96)	0.64 (1.1)	0.002 (0.23, 0.99)
MUAC z score Mean (SD)	0.49 (0.97)	0.04 (1.08)	0.02 (0.07, 0.83)
TSF z score Mean (SD)	0.64 (0.77)	0.06 (1.08)	0.001 (0.24, 0.93)
Truncated Denver development score Median (range)	4 (3-5)	4 (3-6)	0.548

Table 20b: Effect of maternal supplementation with micronutrient enriched paste on growth and development of the breastfed infant at 6 months

Growth and development parameter	Breastfeeding control (n=58)	Breastfeeding supplemented (n=57)	p value for the difference between the groups (95% CI)
Weight kg Mean (SD)	8.35 (1.14)	7.89 (1.04)	0.027 (0.05, 0.86)
Length cm Mean (SD)	66.34 (2.54)	65.99 (2.85)	0.483 (-0.64, 1.35)
Head circumference cm Mean (SD)	43.49 (1.64)	43.74 (1.39)	0.376 (-0.82, 0.31)
MUAC cm Mean (SD)	14.81 (1.26)	14.39 (1.33)	0.082 (-0.06, 0.9)
TSF mm Median (IQR)	10.88 (10-12.31)	10 (9-11.5)	0.015
BMI kg/m^2 Mean (SD)	18.9 (1.85)	18.1 (1.88)	0.024 (0.11, 1.48)
Weight for age z scores Mean (SD)	0.61 (1.17)	0.15 (1.13)	0.035 (0.03, 0.88)
Length for age z score Mean (SD)	-0.35 (1.03)	-0.47 (1.24)	0.574 (-0.3, 0.54)
Head circumference for age z score Mean (SD)	0.42 (1.19)	0.66 (1.03)	0.267 (-0.64, 0.18)
BMI for age z score Mean (SD)	1.07 (1.14)	0.56 (1.2)	0.021 (0.08, 0.94)
Weight for length z score Mean (SD)	1.15 (1.11)	0.67 (1.16)	0.026 (0.06, 0.9)
MUAC z score Mean (SD)	0.62 (1.07)	0.25 (1.17)	0.076 (-0.04, 0.79)
TSF z score Median (IQR)	0.95 (0.48-1.6)	0.52 (-0.09-1.23)	0.013
Truncated Denver development score Median(range)	3 (3-8)	3 (3-6)	0.283

Table 20c: Effect of maternal supplementation with micronutrient enriched paste on growth and development of the breastfed infant at 9 months

Anthropometric measures	Breastfeeding control (n=63)	Breastfeeding supplemented (n=66)	P value for the difference between the groups
Weight kg Mean (SD)	9.42 (1.33)	8.99 (1.21)	0.085 (-0.06, 0.91)
Length cm Mean (SD)	70.63 (3.39)	70.34 (2.87)	0.640 (-0.92,1.49)
Head circumference cm Mean (SD)	45.07 (1.8)	45.41 (1.41)	0.274 (-0.96, 0.27)
MUAC cm Mean (SD)	15.3 (1.31)	14.82 (1.24)	0.056 (-0.01, 0.96)
TSF mm Mean (SD)	10.75 (2.09)	9.7 (1.91)	0.008 (-0.28, 1.81)
BMI Mean (SD)	18.83 (1.93)	18.13 (1.91)	0.065 (-0.04, 1.43)
Weight z scores Mean (SD)	0.65 (1.16)	0.28 (1.15)	0.098 (-0.07, 0.82)
Length for age z score Mean (SD)	0.39 (1.14)	0.41 (1.17)	0.899 (-0.42, 0.47)
Head circumference for age z score Mean (SD)	0.51 (1.02)	0.68 (1.01)	0.368 (-0.57, 0.21)
BMI for age z score Mean (SD)	1.17 (1.17)	0.69 (1.23)	0.042 (0.02, 0.94)
Weight for length z score Mean (SD)	1.18 (1.14)	0.72 (1.19)	0.041 (0.02, 0.92)
MUAC z score Mean (SD)	0.79 (1.05)	0.38 (1.05)	0.046 (0.01, 0.82)
TSF z score Median (IQR)	1.32 (0.47-1.75)	0.74 (-0.07-1.3)	0.004
Truncated Denver development score Median(range)	3 (3-8)	3 (3-6)	0.957

3.7.7.2 Effect of duration of supplementation on the growth and development of the child

The supplemented group was further analysed for intragroup differences as per duration of supplementation/breastfeeding. There were significant differences between the two groups in the infants of the mothers with a shorter duration of supplementation had lower measurements for all the indices, more especially for weight, length, weight for age and length for age z scores (see table 20d).

Table 20d: Sub group analysis: Effect of duration of nutritional supplementation on the growth and development of the child

		Supplementation		p value for the difference between the groups (95% CI)
		≤ 3.5 months (n=21)	>3.5 months (n=45)	
Weight kg Mean (SD)	**2 week**	3.38 (0.4)	3.79 (0.46)	0.001 (-0.64, -0.17)
	14 week	5.88 (0.74)	6.61 (0.78)	0.003 (-1.2, -0.25)
	6 month	7.47 (0.83)	8.09 (1.08)	0.037 (-1.18, -0.04)
Length cm Mean (SD)	**2 week**	49.87 (2.19)	51.55(1.65)	0.001 (-2.65, -0.71)
	14 week	59.15 (2.7)	60.93 (2.31)	0.020 (-3.27, -0.29)
	6 month	64.96 (2.57)	66.46 (2.88)	0.064 (-3.1, 0.09)
Head circumference cm Mean (SD)	**2 week**	35.93 (1)	36.69 (1.2)	0.014 (-1.37, -0.16)
	14 week	40.68 (1.28)	41.53 (1.43)	0.054 (-1.71, 0.02)
	6 month	43.42 (1.06)	43.89 (1.51)	0.244 (-1.26, 0.33)
MUAC cm Mean (SD)	**2 week**	10.25 (0.94)	10.7 (0.89)	0.064 (-0.93, 0.03)
	14 week	13.04 (1.32)	13.61 (0.97)	0.086 (-1.23, 0.08)
	6 month	13.84 (1.16)	14.64 (1.34)	0.034 (-1.53, -0.06)
TSF mm Mean (SD)	**2 week**	5.6 (1.35)	6.59 (1.48)	0.013 (-1.77, -0.22)
	14 week	9.02 (1.8)	10.27 (1.52)	0.014 (-2.24, -0.27)
	6 month	9.61 91.8)	10.4 (1.82)	0.135 (-1.82, 025)
BMI kg/m^2 Mean (SD)	**2 week**	13.57(0.95)	14.24 (1.45)	0.060 (-1.36, 0.03)
	14 week	16.8 (1.74)	17.78 (1.58)	0.055 (-1.98, 0.02)
	6 month	17.69 (1.48)	18.29 (2.03)	0268 (-1.67, 0.47)
Weight for age z score Mean (SD)	**2 week**	-0.74 (0.86)	0.03 (0.8)	0.001 (-1.2, -0.33)
	14 week	-0.77 (1.18)	0.34 (0.92)	0.001 (-1.72, -0.49)
	6 month	-0.44 (1.09)	0.42 (1.06)	0.006 (-1.47, -0.25)
Length for age z score Mean (SD)	**2 week**	-1.21 (1.17)	-0.36 (0.82)	0.001 (-1.35, -0.35)
	14 week	-1.24 (1.32)	-0.23 (0.94)	0.003 (-1.66, -0.36)
	6 month	-1.11 (1.27)	-0.17 (1.13)	0.007 (-1.61, -0.27)
Head circumference for age z score Mean (SD)	**2 week**	0.17 (1.06)	0.8 (0.91)	0.014 (-1.15, -0.13)
	14 week	0.09 (1.16)	0.94 (1)	0.010 (-1.5, -0.21)
	6 month	0.24 (0.92)	0.85 (1.03)	0.038 (-1.17, -0.04)
BMI for age z score Mean (SD)	**2 week**	-0.13 (0.79)	0.35 (1.06)	0.069 (-1, 0.04)
	14 week	-0.08 (1.27)	0.64 (1.01)	0.036 (-1.39, -0.05)
	6 month	0.29 (0.98)	0.69 (1.27)	0.244 (-1.09, 0.281)
Weight for length z score Mean (SD)	**2 week**	0.15 (0.92)	0.23 (1.23)	0.782 (-0.69, 0.52)
	14 week	0.34 (1.29)	0.75 (1.02)	0.233 (-1.08, 0.27)
	6 month	0.43 (0.94)	0.79 (1.25)	0.275 (-1.03, 0.3)
MUAC for age z score Mean (SD)	**14 week**	-0.44 (1.39)	0.21 (0.91)	0.054 (-1.29, 0.01)
	6 month	-0.3 (1.1)	0.5 (1.13)	0.016 (-1.44, -0.15)
TSF for age z score Mean (SD)	**14 week**	-0.55 (1.36)	0.26 (0.90)	0.014 (-1.46, -0.17)
	6 month	0.17 (1.09)	0.61 (0.94)	0.122 (-1.01, 0.122)
Truncated Denver developmental score Median (range)	**14 week**	4 (4-6)	4 (3-6)	0.048
	6 month	3 (3-6)	3 (3-5)	0.014

Figure 23: Mean weight for age z scores per duration of supplement in the supplemented group

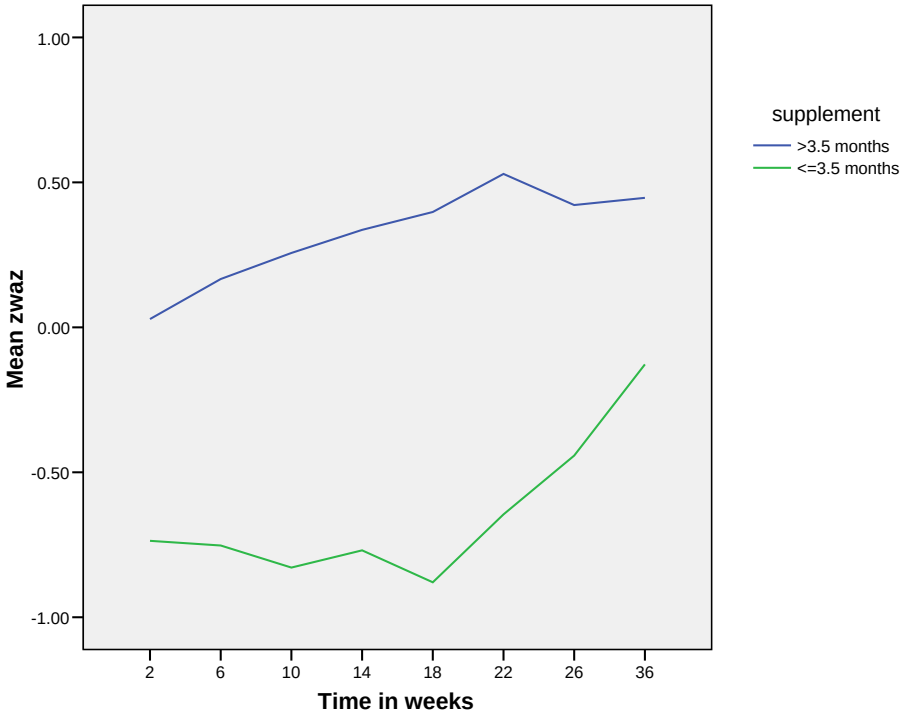


Figure 24: Mean length for age z scores per duration of supplement in the supplemented group

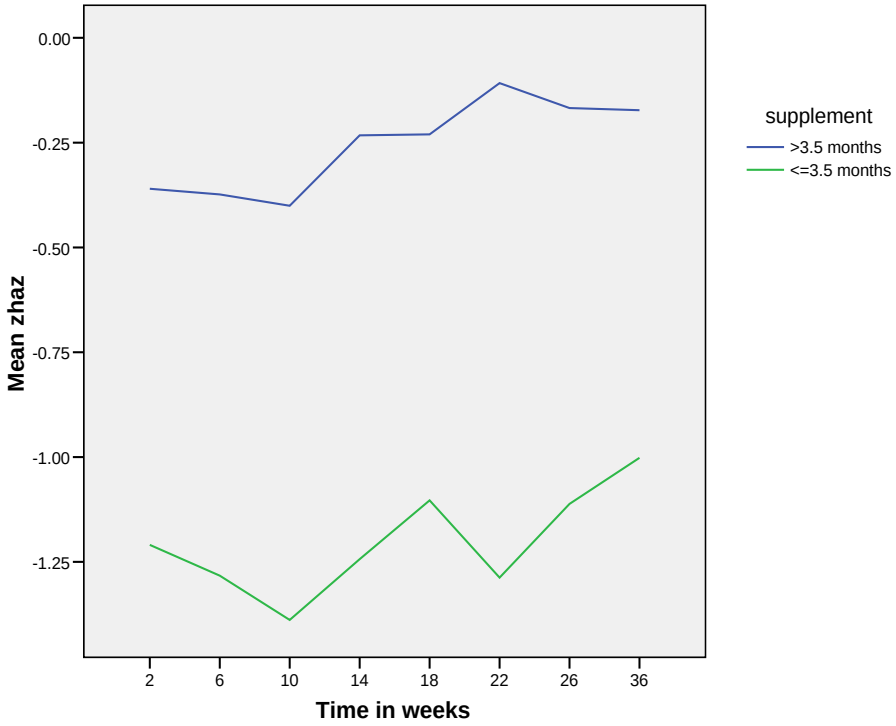


Figure 25: Mean weight for length z scores per duration of supplement in the supplemented group

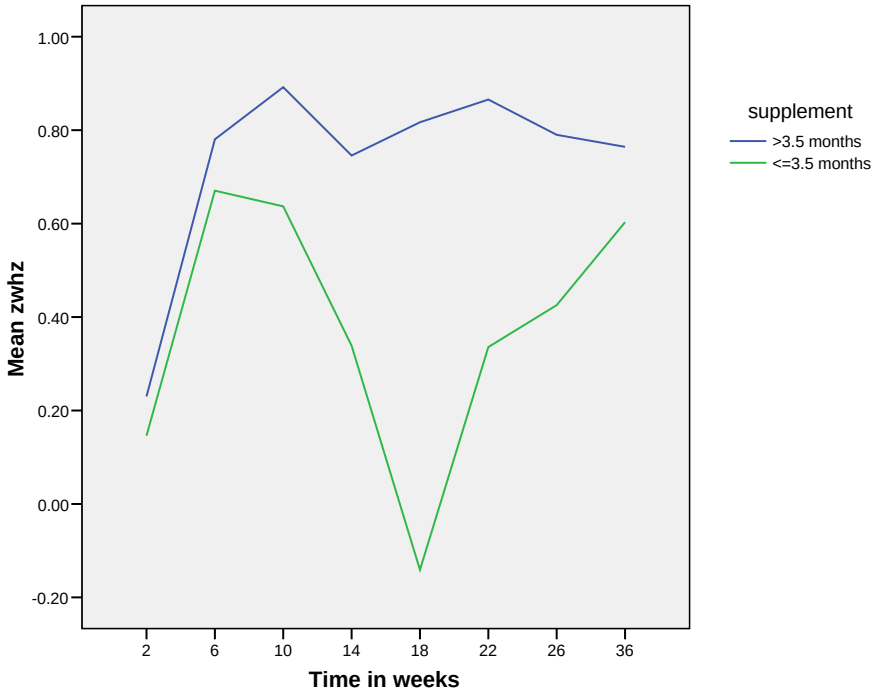
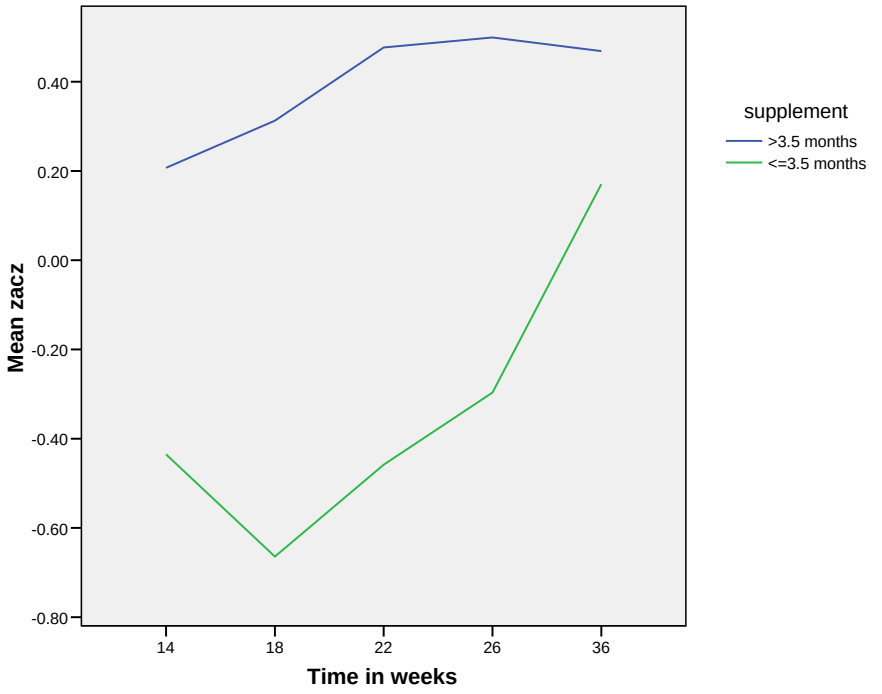


Figure 26: Mean MUAC for age z scores per duration of supplement in the supplemented group



3.7.7.3 Longitudinal analyses of the growth

Generalised estimating equations with robust errors and an exchangeable covariance matrix were used to analyse the growth adjusted for mother's CD4 count, BMI and duration of breastfeeding. There was a significant increase in all the measurements with time in both groups except the z score for head circumference for age where there were no significant differences noted. The TSF/age z score was lower in the breastfeeding supplemented group over time. Lower breastfeeding duration (≤ 3.5 months) was significantly associated with lower measurements in all variables except the z scores for head circumference for age and weight for length. There was a significant association of increased weight with mother's BMI. There was no effect of group time interaction in any of the parameters (table 20e-20q, appendix19).

3.7.8 Effect of maternal nutritional supplementation with incidence of opportunistic infections in the breastfed infant

3.7.8.1 Cross-sectional analysis

2-14 weeks: No significant differences between the two groups for the incidence rate of any of the infections; (see table 21a).

14 weeks to 6 months: No significant differences between the two groups; (see table 21b).

6 months to 9 months: No significant differences between the two groups for the incidence rate of any of the infections; (see table 21c).

Table 21a: Effect of maternal nutritional supplementation on incidence of opportunistic infections in the breastfed infant per 1000 child-week of follow-up between 2 and 14 weeks of age

Clinical conditions/events	Breastfeeding Control (n=63)		Breastfeeding supplemented (n=66)		Incidence rate ratio (95% CI)	P value
	Count	Incidence rate	Count	Incidence rate		
Oral thrush	13	14.74	23	24.89	1.69 (0.86-3.31)	0.130
Diarrhoea	6	6.8	8	8.66	1.27 (0.44-3.66)	0.668
Ear discharge	1	1.13	0	0	n/a	0.488
LRTI	0	0	3	3.25	n/a	0.134
TB	0	0	0	0	n/a	n/a
FTT	0	0	1	1.08	n/a	0.512
Skin sepsis	7	7.94	9	9.74	1.23 (0.46-3.29)	0.695
Meningitis	0	0	0	0	n/a	n/a
Delay in milestones	0	0	2	2.16	n/a	0.262
Admissions	0	0	1	1.08	n/a	0.512
Death	0	0	0	0	n/a	n/a

Table 21b: Effect of maternal nutritional supplementation on the incidence of opportunistic infections in the breastfed infant per 1000 child-week of follow-up between 14 weeks to 6 months of age

Clinical conditions/events	Breastfeeding Control (n=63)		Breastfeeding supplemented (n=66)		Incidence rate ratio (95% CI)	P value
	Count	Incidence rate	Count	Incidence rate		
Oral thrush	6	7.94	6	7.57	0.95 (0.31-2.96)	0.937
Diarrhoea	14	18.52	20	25.25	1.36 (0.69-2.69)	0.379
Ear discharge	4	5.29	2	2.52	0.48 (0.09-2.51)	0.421
LRTI	2	2.64	4	5.05	1.91 (0.36-10.12)	0.487
TB	5	6.61	1	1.26	0.19 (0.03-1.3)	0.112
FTT	2	2.64	5	6.31	2.39 (0.49-11.69)	0.317
Skin sepsis	1	1.32	6	7.58	5.73 (0.88-37.18)	0.08
Meningitis	0	0	1	1.26	n/a	0.512
Delay in milestones	4	5.29	9	11.36	2.15 (0.68-6.78)	0.207
Admissions	2	2.65	6	7.58	2.86 (0.62-13.21)	0.201
Death	0	0	1	1.26	n/a	0.512

Table 21c: Effect of maternal nutritional supplementation on the incidence of opportunistic infections in the breastfed infant per 1000 child-week of follow-up between 6 to 9 months of age

Clinical conditions/events	Breastfeeding Control (n=63)		Breastfeeding supplemented (n=66)		Incidence rate ratio (95% CI)	P value
	Count	Incidence rate	Count	Incidence rate		
Oral thrush	2	4.37	0	0	0	0.168
Diarrhoea	13	28.38	16	24.24	0.85 (0.41-1.77)	0.672
Ear discharge	0	0	0	0	n/a	n/a
LRTI	2	4.37	0	0	0	0.168
FTT	0	0	1	1.52	n/a	0.590
TB	2	4.37	0	0	0	0.168
Skin sepsis	1	2.18	1	1.52	0.69 (0.04-10.93)	0.819
Meningitis	1	2.18	0	0	0	0.410
Delayed milestones	2	4.37	2	3.03	0.69 (0.1-4.87)	0.732
Admissions	1	2.18	1	1.52	0.69 (0.04-10.93)	0.819
Death	2	4.37	2	3.03	0.69 (0.1-4.87)	0.732

3.7.8.2 Effect of duration of breastfeeding/maternal supplementation on disease progression in the infants of the supplemented mothers

On assessing for intragroup differences within the supplemented group, there were significantly higher incidence rates of oral thrush in the infants of the mothers who took the supplement for 3.5 months or less.

Table 21d: Effect of duration of maternal supplementation on disease progression in the infants of the supplemented mothers (2 weeks-6 Months); incidence rates per 1000 child-week of follow-up

Clinical conditions/events	Duration of issue of supplement				Incidence rate ratio (95% CI)	P value
	≤ 3.5 months (n=21)		> 3.5 months (n=45)			
	Count	Incidence rate	Count	Incidence rate		
Oral thrush	15	30.12	14	12.09	2.49 (1.23-5.04)	0.016
Ear discharge	1	2.01	1	0.86	2.33 (0.16-34.3)	0.601
Diarrhoea	10	20.08	18	15.54	1.29 (0.6-2.79)	0.514
LRTI	4	8.03	3	2.59	3.1 (0.75-12.83)	0.156
FTT	0	0	6	5.18	n/a	0.117
TB	1	2.01	0	0	n/a	0.301
Skin sepsis	7	14.06	7	6.04	2.33 (0.84-6.43)	0.126
Meningitis	1	2.01	0	0	n/a	0.301
Delayed milestones	4	8.03	3	2.59	3.1 (0.75-12.83)	0.156
Admissions	4	8.03	3	2.59	3.1 (0.75-12.83)	0.156
Death	1	2.01	1	0.86	2.33 (0.16-34.3)	0.601

3.7.8.3 Longitudinal analysis

Generalised linear model with Poisson distribution and log function was used to analyse the data further adjusted for duration of breastfeeding. There were no differences between the groups for any of the events. However there was a significant positive impact of breastfeeding duration on the event of death (IRR 10.68; 95% CI 1.05, 108.25); delayed milestones (IRR 8.14; 95%CI 2.86, 23.17); hospital admission (IRR 7.36; 95% CI 1.89-28.65); LRTI (IRR 5.22; 95% CI 1.47, 18.51); and skin sepsis (IRR 2.44 95% CI 1.07, 5.39). The longer the infant was breastfed was seen to be significantly protective for the diagnosis of these events (table 21e-21o, appendix 20).

3.7.9 Effect of maternal nutritional supplementation on the growth and development of the breastfed infected infants

3.7.9.1 Cross-sectional analyses

There were no significant differences between the infants in both groups for growth parameters at any time point (table 22a). The truncated Denver developmental score was similar in both groups.

Table 22a: Growth and development over six months for the breastfed infants who were HIV positive

Growth parameters		Breastfeeding control (n=5)	Breastfeeding supplemented (n=8)	P value for the difference between the groups (95% CI)
Weight/Age z score Mean (SD)	**2 week**	-0.52 (0.9)	-0.79 (0.89)	0.606 (-0.85. 1.39)
	14 week	-0.31 (1.54)	-1.58 (1.74)	0.285 (-1.32, 3.39)
	6 month	-1.07 (1.57)	-1.52 (0.95)	0.570 (-1.28, 2.18)
Length/Age z score Mean (SD)	**2 week**	-1.28 (1.05)	-1.15 (1.06)	0.843 (-1.45, 1.21)
	14 week	-1.09 (0.97)	-1.47 (1.24)	0.627 (-1.36, 2.11)
	6 month	-1.5 (0.78)	-2.26 (0.83)	0.156 (-0.35, 1.86)
H C /Age z score Mean(S D)	**2 week**	0.36 (0.98)	-0.25 (0.98)	0.334 (-0.73, 1.94)
	14 week	0.42 (1.12)	-1.08 (1.1)	0.084 (-0.26, 3.26)
	6 month	-0.25 (1.77)	-0.8 (0.55)	0.487 (-1.16. 2.26)
BMI/ Age z score Mean (SD)	**2 week**	0.23 (0.85)	-0.26 (0.61)	0.243 (-0.39, 1.38)
	14 week	0.43 (1.52)	-1.08 (1.56)	0.185 (-0.93, 3.96)
	6 month	-0.27 (1.73)	-0.26 (0.67)	0.982 (-1.74, 1.7)
Weight/Length z score Mean SD)	**2 week**	0.55 (0.81)	-0.02 (0.53)	0.151 (-0.24, 1.38)
	14 week	0.78 (1.31)	-0.59 (1.12)	0.144 (-0.59, 3.32)
	6 month	-0.1 (1.67)	-0.02 (0.6)	0.922 (-1.71, 1.57)
MUAC for age z score Mean (SD)	**14 week**	-0.02 (1.15)	-1.15 (2.3)	0.366 (-1.63, 3.88)
	6 month	-1.2 (1.35)	-0.53 (1.19)	0.406 (-2.39, 1.06)
TSF for age z score Mean (SD)	**14 week**	0.28 (0.59)	-0.95 (2.24)	0.269 (-1.2, 3.67)
	6 month	-0.02 (1.04)	-0.06 (1)	0.947 (-1.35, 1.44)
Truncated Denver developmental score Median (range)	**14 week**	4 (4-4)	3 (3-7)	0.476
	6 months	3 (3-8)	4.5 93-6)	0.610

3.7.9.2 Longitudinal analysesof growth and development in the HIV positive breastfed infants (table 22b - 22 n, appendix 21)

Generalised estimating equations were used to analyse the longitudinal data adjusted for duration of breastfeeding. There were no significant differences between the groups. Weight, length, Head circumference, MUAC and BMI increased with time equally in both groups. The MUAC, TSF, BMI measurements and the TSF/age; MUAC/age; weight/length; BMI/age z scores were significantly higher in the infants who breastfed for longer than 3.5 months. There was also a trend for a higher weight/age in the infants' breastfeeding for longer; this was of borderline significance. The length/age and head circumference/age z scores were not significantly different over time.

3.7.10 Effect of nutritional supplementation in the incidence of opportunistic infections in the breastfed infected child

3.7.10.1 Cross-sectional analyses

2 to 14 weeks: There were no significant differences between the two groups for any of the incidence rates (see table 23a).

14 weeks to 6 months: There was a significant difference between the incidence rates of seborrhaeic dermatitis between the control group which had four episodes and the supplemented group which had no episodes (p=0.043); there were no other significant differences between the two groups (see table 23b).

6 months to 9 months: There were no significant differences between the two groups for any of the incidence rates (see table 23c).

Table 23 a. Effect of maternal nutritional supplementation in the incidence of opportunistic infections in the breastfed infected child 2 weeks to 14 weeks of age per 1000 child-week of follow-up

Clinical conditions/events	Breastfeeding Control (n=5)		Breastfeeding supplemented (n=6)		Incidence rate ratio (95% CI)	P value
	Count	Incidence rate	Count	Incidence rate		
Oral thrush	2	28.57	5	59.52	2.08 (0.42-10.36)	0.406
Diarrhoea	1	14.29	1	11.9	0.83 (0.05-13.27)	0.909
Ear discharge	1	14.29	0	0	0	0.454
LRTI	0	0	0	0	0	n/a
TB	0	0	0	0	0	n/a
FTT	0	0	0	0	0	n/a
Seborrhaeic dermatitis	4	57.14	0	0	0	0.043
Skin sepsis	0	0	1	11.9	n/a	0.546
Meningitis	0	0	0	0	0	n/a
Delayed milestones	0	0	1	11.9	n/a	0.546
Admissions	0	0	0	0	0	n/a
Death	0	0	0	0	0	n/a

Table 23 b. Effect of maternal nutritional supplementation in the incidence of opportunistic infections in the breastfed infected child 14 weeks to 6 months of age per 1000 child-week of follow up

Clinical conditions/events	Breastfeeding Control (n=5)		Breastfeeding supplemented (n=7)		Incidence rate ratio (95% CI)	P value
	Count	Incidence rate	Count	Incidence rate		
Oral thrush	2	33.3	1	11.9	0.36 (0.04-3.55)	0.449
Diarrhoea	2	33.3	1	11.9	0.36 (0.04-3.55)	0.449
Ear discharge	1	16.67	1	11.9	0.71 (0.04-11.27)	0.833
LRTI	2	33.3	4	47.62	1.43 (0.26-7.73)	0.718
FTT	2	33.3	1	11.9	0.36 (0.04-3.55)	0.449
TB	4	66.67	2	23.81	0.36 (0.07-1.81)	0.252
Seborrhaeic dermatitis	2	33.3	0	0	0	0.174
Skin sepsis	0	0	2	23.81	n/a	0.340
Meningitis	0	0	0	0	0	n/a
Retroviral encephalopathy	0	0	4	47.62	n/a	0.116
Delayed milestones	1	16.67	6	71.43	4.28 (0.62-29.86)	0.161
Admissions	1	16.67	4	47.62	2.86 (0.35-23.19)	0.376
Death	0	0	1	11.9	n/a	0.583

Table 23 c. Effect of maternal nutritional supplementation in the incidence of opportunistic infections in the breastfed infected child 6 months to 9 months of age per 1000 child-week of follow-up

Clinical conditions/events	Breastfeeding control (n=5)		Breastfeeding supplemented (n=7)		Incidence rate ratio (95% CI)	P value
	Count	Incidence rate	Count	Incidence rate		
Oral thrush	1	20	0	0	0	0.417
Diarrhoea	2	40	1	14.29	0.36 (0.04-3.55)	0.449
Ear discharge	0	0	0	0	n/a	n/a
LRTI	1	20	0	0	0	0.417
FTT	0	0	0	0	n/a	n/a
TB	1	20	0	0	0	0.417
Seborrhaeic dermatitis	0	0	0	0	0	n/a
Skin sepsis	0	0	0	0	n/a	n/a
Meningitis	1	20	0	0	0	0.417
Retroviral encephalopathy	0	0	2	28.6	n/a	n/a
Delay in milestones	1	20	2	28.6	1.43 (0.13-15.56)	0.822
Admissions	1	20	0	0	0	0.417
Death	2	40	1	14.29	0.36 (0.04-3.55)	0.449

3.7.10.2 Longitudinal analyses for the incidence of opportunistic infections in both groups (table 23d-23m, appendix 22)

There were no significant differences between the two groups or any effect of the duration of breastfeeding. There was a lower incidence of seborrhaeic dermatitis amongst the infants of the supplemented mothers which was of borderline significance (p=0.057).

3.7.11 Effect of nutritional supplement on selected micronutrients in the breastmilk of breastfeeding mothers at 6 months

3.7.11.1 Effect of nutritional supplement on selected micronutrients in the breastmilk

There were no significant differences between the two groups with regards to changes in the micronutrient levels in the breastmilk; (see table 24a-b).

Table 24a: Mean levels of selected micronutrients in the breastmilk of breastfeeding mothers at baseline and 26 weeks

Micronutrient level Mean (SD)		**Breastfeeding Control (n=26)**	**Breastfeeding supplemented(n=27)**	**P value for the difference between the groups (95% CI)**
Zinc µg/dL	2 weeks	367.15 (118.86)	327.39 (78.44)	0.155 (-15.58, 95.08)
	6 months	96.55 (63.53)	98.88 (62.95)	0.896 (-37.92, 33.28)
Retinol SI µmol/L	2 weeks	2.91 (1.29)	3.06 (1.36)	0.683 (-0.89, 0.59)
	6 months	2.36 (1.23)	2.05 (1.06)	0.345 (-0.34, 0.95)

Table 24b: Effect of nutritional supplementation on selected micronutrients in the breastmilk of breastfeeding mothers from baseline to 6 months

Change in parameter over 6 months from baseline	**Breastfeeding Control (n=26)**	**Breastfeeding supplemented(n=24)**	**P value for the difference between the groups (95% CI)**
Zinc µg/dL Mean (SD)	-270.59 (106.93)	-230.76 (65.71)	0.123 (-90.81, 11.15)
Retinol SI µmol/L Mean SD)	-0.48 (1.69)	-0.95 (1.77)	0.345 (-0.53, 1.48)

3.7.11.2 Sub-group analysis of the effect of nutritional supplement on selected micronutrients in the breastmilk of breastfeeding mothers at 26 weeks by BMI category

The difference between the groups was not significant for any of the changes in the micronutrient levels in the breastmilk within the subgroup analysis; (see table 24c).

Table 24c: Sub-group analysis of the effect of nutritional supplement on selected micronutrients in the breastmilk of breastfeeding mothers at 26 weeks (by BMI category)

Change in parameter over 6 months	Low to normal BMI (≤24.99 kg/m^2)			High BMI (≥ 25 kg/m^2)		
	Breastfeeding Control (n=10)	Breastfeeding Supplemented (n=11)	p value for the difference between the groups (95% CI)	Breastfeeding Control (n=16)	Breastfeeding Supplemented (n=13)	p value for the difference between the groups (95% CI)
Zinc µg/dL Mean (SD)	-259.17 (146.21)	-235.2 (68.24)	0.630 (-126.53, 78.59)	-277.73(78.02)	-227.01(66.04)	0.073 (-106.61, 5.16)
Retinol SI µmol/L Mean (SD)	0.05 (1.87)	-0.41 (1.62)	0.551 (-1.13, 2.06)	-0.88 (1.47)	-1.38 (1.83)	0.446 (-0.82, 1.82)

3.7.12 Effect of nutritional supplement on the macronutrient composition of the breastmilk

3.7.12.1 Effect of nutritional supplementation on the macronutrient composition of breastmilk at 2, 14 and 26 weeks

The changes in the breastmilk were similar in both groups with no significant differences in between the groups for the changes at any time point; (see table 25a).

Note: The micronutrients and macronutrients in the breastmilk could not be analysed per duration of supplementation/breastfeeding as the issue of supplement stopped on cessation of breastfeeding, hence samples were not collected as mothers had stopped breastfeeding before their 14 week visit.

Table 25a: Effect of nutritional supplement on the macronutrient composition of the breastmilk of breastfeeding mothers at 2, 14 and 26 weeks

Macronutrient	Breastfeeding control			Breastfeeding supplemented			P value for the difference between the groups (95% CI)		
	2 week (n=59)	14 week (n=22)	6 month (n=28)	2 week (n=60)	14 week (n=22)	6 month (n=26)	2 weeks	14 weeks	6 months
Fat g/dL Mean (SD)	4.89 (1.39)	3.95 (1.44)	4.46 (1.42)	4.9 (1.69)	4.37 (1.49)	4.21 (1.65)	0.972 (-0.57, 0.55)	0.343 (-1.31, 0.47)	0.560 (-0.59, 1.09)
Lactose g/dl Mean (SD)	6.31 (0.46)	5.97 (0.68)	5.67 (1.02)	6.22 (0.6)	5.47 (1.07)	5.94 (0.69)	0.359 (-0.10, 0.28)	0.071 (-0.05, 1.05)	0.268 (-0.75, 0.21)
Solids g/dL Mean (SD)	13.17 (1.2)	11.58 (1.42)	11.73 (1.47)	13.21 (1.91)	11.41 (1.66)	11.5 (1.38)	0.899 (-0.62, 0.54)	0.705 (-0.76, 1.12)	0.551 (-0.55, 1.01)
Energy kcal/dl Mean (SD)	77.14 (11.5)	65.59 (12.77)	68.6 (12.5)	76.5 (13.4)	67.14 (13.2)	66.3 (13.18)	0.793 (-3.94, 5.15)	0.695 (-9.45, 6.36)	0.499 (-4.63, 9.38)
Protein g/dL Median (IQR)	1.6 (1.4-1.8)	1.1 (0.9-1.3)	1 (0.8-1.3)	1.5 (1.3-1.8)	1 (0.8-1.2)	0.9 (0.8-1.1)	0.393	0.320	0.083

Figure 27: Mean and 95% Confidence intervals of the protein content in breastmilk over time

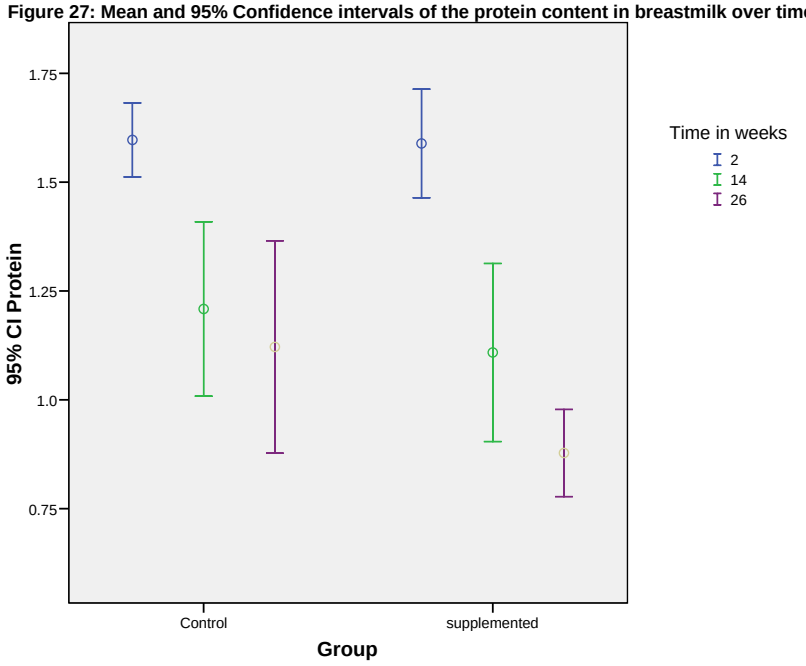


Figure 28: Mean and 95% Confidence intervals of the lactose content in breastmilk over time

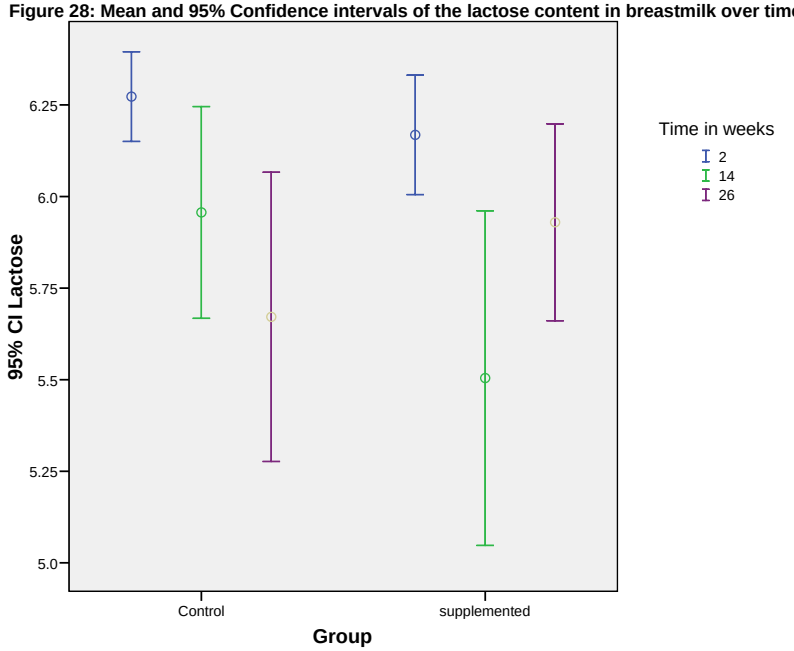


Figure 29: Mean and 95% Confidence intervals of the fat content in breastmilk over time

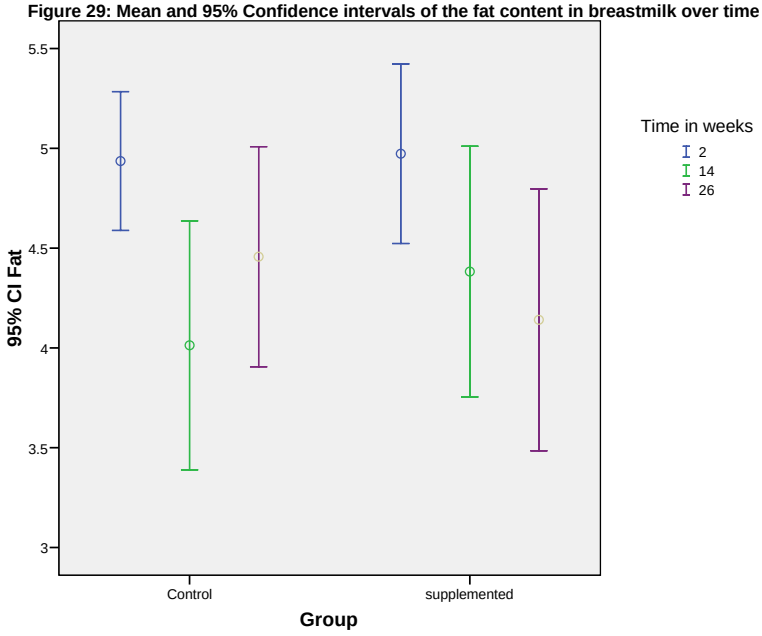
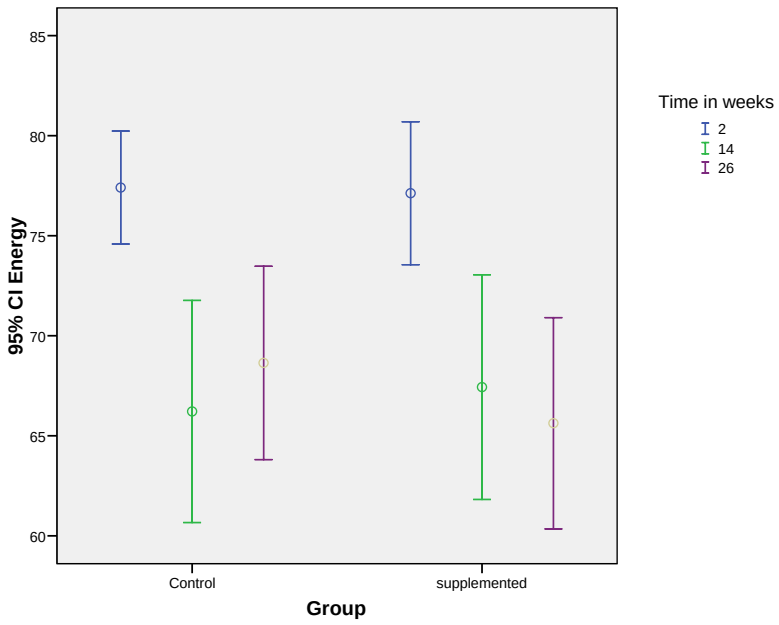


Figure 30: Mean and 95% Confidence intervals of the energy content in the breastmilk over time



3.7.12.2 Sub-analysis of the effect of nutritional supplement on the macronutrient composition of the breastmilk of breastfeeding mothers by BMI category

There were similar changes in breastmilk composition over time in both groups; there were no significant differences between the two groups; (table 25b)

Table 25b: Sub-analysis of the effect of nutritional supplement on the macronutrient composition of the breastmilk of breastfeeding mothers at 2, 14 and 26 weeks (by BMI category)

Low to Normal BMI

Macronutrient	Breastfeeding control			Breastfeeding supplemented			p value for the difference between the groups (95% CI)		
	2(n=23)	14(n=10)	26(n=11)	2(n=29)	14(n=11)	26(n=11)	2	14	26
Fat g/do Mean (SD)	4.53 (1.12)	4.1 (1.73)	4.69 (0.98)	4.814 (1.47)	4.1 (1.40)	4.63 (2.04)	0.44 (-1, 0.46)	1 (-1.4,1.4)	0.93 (-1.4,1.49)
Lactose g/dL Mean (SD)	6.45 (0.33)	5.79 (0.89)	5.32 (1.35)	6.26 (0.62)	5.418 (0.99)	5.82 (0.74)	0.18 (-0.1,0.48)	0.38 (-0.5,1.2)	0.3 (-1.5, 0.5)
Solids g/dL Mean (SD)	13.02 (1.06)	11.710 (1.60)	11.78 (1.23)	13.32 (2.21)	11.23 (1.34)	11.76 (1.64)	0.55 (-1.3, 0.7)	0.46 (-0.9,1.8)	0.98 (-1.3, 1.3)
Energy kcal/dl Mean (SD)	74.78 (9.65)	67 (14.77)	70.27 (9.03)	75.83 (10.93)	65.18 (11.88)	69.27 (15.89)	0.46 (-0.9, 1.8)	0.98 (-1.3,1.3)	0.72 (-6.9, 4.8)
Protein g/dL Median (IQR)	1.6 (1.5-1.8)	1.1 (1.1-1.4)	1.1 (0.9-1.6)	1.5 (1.2-1.8)	1.1 (0.9-1.4)	0.9 (0.6-1.1)	0.22	0.28	0.12

High BMI

Macronutrient	Breastfeeding control			Breastfeeding supplemented			p value for the difference between the groups (95% CI)		
	2(n=36)	14(n=12)	26(n=17)	2(n=31)	14(n=11)	26(n=15)	2	14	26
Fat g/dL Mean (SD)	5.12 (1.51)	3.82 (1.21)	4.31 (1.66)	4.98 (1.89)	4.64 (1.58)	3.91 (1.29)	0.74 (-0.7, 1)	0.18 (-2,0.4)	0.46 (-0.7, 1.5)
Lactose g/dL Mean (SD)	6.23 (0.52)	6.13 (0.43)	5.9 (0.68)	6.19 (0.58)	5.53 (1.19)	6.03 (0.66)	0.8 (-0.2, 0.3)	0.12 (-0.2,1.4)	0.6 (-0.6, 0.4)
Solids g/dL Mean (SD)	13.27 (1.29)	11.48 (1.31)	11.69 (1.64)	13.11 (1.61)	11.58 (1.97)	11.29 (1.17)	0.65 (-0.6, 0.9)	0.88 (-1.5,1.3)	0.44 (-0.6, 1.4)
Energy kcal/dl Mean (SD)	78.64 (12.44)	64.42 (11.39)	67.59 (14.42)	77.19 (15.59)	69.09 (14.73)	64.07 (10.85)	0.67 (-5.4, 8.3)	0.40 (-16, 6.7)	0.45 (-5.8, 12.8)
Protein g/dL Median (IQR)	1.55 (1.4-1.8)	1 (0.73-1.3)	0.9 (0.75-1.1)	1.5 (1.4-1.9)	1 (0.6-1.2)	0.9 (0.8-1)	0.94	0.52	0.36

3.8 Cervical screening in the mothers (table 26)

Cervical screening was done in 87 (42.6%) of the mothers; 22 (25.3%) of the cervical smears were normal; 14.9% smears were suggestive of vaginal candidiasis; 28.7% suggestive of bacterial vaginosis; 23% cervical smears had changes suggestive of Human papilloma virus infection (HPV) with a low grade squamous intraepithelial lesion; 4.6% with a high grade squamous intraepithelial lesion; 2.3% with Trichomonas vaginalis; and (1.1%) with abnormal squamous cell of undetermined significance.

Table 26: Cervical smear cytology

Cervical smear cytology	Formula feeding (n=33)	Breastfeeding control (n=28)	Breastfeeding supplemented (n=26)
NAD	8	8	6
Vaginal Candidiasis	5	6	2
Bacterial vaginosis	11	5	9
Low grade squamous intraepithelial lesion (CIN1/HPV)	8	6	6
HSIL	1	2	1
Trichomonas vaginalis	0	1	1
ASCUS	0	0	1

3.9 Infant feeding practice (table 27)

Of the 75 mothers who had chosen to formula-feed, 74 (98.7%) started with formula at birth; one mother breastfed for a week after delivery so as to not reveal her status and then stopped. Most families/partners (95.6%) were supportive of the mother's choice of feeding mode. When assessed for AFASS criteria, only 38.7% of the formula-feeding mothers actually fulfilled the criteria.

Of the breastfeeding infants, 47 (34%) were put to breast in the 1st hour of delivery. Amongst the reasons for stopping breastfeeding before 6 months, returning to work was the most frequent (12.4%). Other reasons were breast pathology (8.5%); fear of mixed feeding (6.2%); insufficient breastmilk (5.4%); pressure from family/health care worker (1.6%); mother unwell (1.6%); and unknown (3.1 %).

Mothers in the breastfeeding control group breastfed their infants longer than the supplemented group (p=0.009). Exclusive breastfeeding rates were 87.4% at 3 months and 57.4% at 6 months (table 27b).

On assessing for factors associated with duration of breastfeeding, access to basic amenities was significant (p=0.014). More mothers with better access to basic amenities continued breastfeeding for longer periods (table 27c).

Table 27a: Summary statistics of breastfeeding groups

Parameters	**Group 2 (n=63)**	**Group3 n=66**	**Mann-Whitney U test (p value)**
Breastfeeding at birth (%)	63 (100)	66 (100)	n/a
Partner/Family supportive of choice (%)	63 (100)	62 (93.9)	n/a
Fulfillment of AFASS criteria	5 (7.9)	5 (7.6)	n/a
Time to breast after delivery in hours Median(range)	3 (0-96)	2 (0-72)	0.08
Other feeds given before commencement of BF (%)	24 (38.1)	30 (53.6)	n/a
Other feeds given in the first 72 hours of life (%)	13 (20.6)	10 (12.5)	n/a
No. of months of Breastfeeding Median (range)	6 (1-9)	5.5 (1-7)	0.009
No. of months of EBF Median(range)	6 (1-6)	5 (0-7)	0.022
No. of months of non exclusive BF Median (range)	0 (0-3)	0 (0-3)	0.469
No. of months of HTEBM Median (range)	0 (0-6)	0	0.018

Table 27b: Duration of exclusive breastfeeding

Duration of EBF (months)	**Breastfeeding control (n=63) (%)**	**Breastfeeding supplemented (n=66) (%)**	**Total (n=129) (%)**
1	**63 (100)**	**65 (98.5)**	**128 (99.2)**
2	**60 (95.2)**	**58 (87.9)**	**118 (91.5)**
3	**58 (92.1)**	**51 (77.3)**	**109 (84.5)**
4	**51 (81)**	**42 (63.6)**	**93 (72.1)**
5	**46 (73)**	**35 (53)**	**81 (62.8)**
6	**42 (66.7)**	**32 (48.5)**	**74 (57.4)**
7	**0 (0)**	**1 (1.5)**	**1 (0.8)**

Table 27c: Factors associated with duration of feeding

Factors		Duration of Breastfeeding		p value (95% CI)
		<=3.5 months	>3.5 months	
Maternal age years Mean (SD)		25.11(4.02	25.49(4.75)	0.679 (-1.42, 2.16)
CD4 cells/µL Median (min/max)		358 (221-1018)	440.5 (225-2922)	0.080
Haemoglobin g/dL Mean (SD)		11.52 (1.63)	11.7 (1.54)	0.566 (-0.44,0.79)
Albumin g/L Mean (SD)		31.14 (4.83)	31.69 (4.04)	0.522 (-1.14,2.23)
Education yrs Median (min/max)		12 (2-15)	11(2-15)	0.158
Food insecurity		12 (29.3)	29 (70.7)	0.432
Access to basic amenities (% within category)	**Good**	5 (11.4)	39 (88.6)	0.014
	Medium	26 (34.7)	49 (65.3)	
	Low	4 (40)	6 (60)	
Parity (% within category)	**Primiparous**	12 (28.6)	30 (71.4)	0.140
	Biparous	17 (34.7)	32 (65.3)	
	Multiparous	6 (15.8)	32 (84.2)	
Income (% within category)	**Self**	7 (23.3)	23 (76.7)	0.822
	Partner	12 (25)	36 (75)	
	Family	13 (32.5)	27 (67.5)	
	Other	3 (27.3)	8 (72.7)	

3.10 ***Status of mothers and infants at the end of follow-up*** *(table 28)*

There were no maternal deaths. 11 (5.4%) of the mothers were started on antiretroviral therapy as they became eligible. 6 (2.9%) of the mothers became pregnant again by 9 months of delivery of their last born. 60(29.4%) of the mothers either went on to gain new or regain their old employment by the end of follow-up.

There were 6 infant deaths (2.9%). All deaths were attributed to gastroenteritis related morbidity. Five deaths were amongst the vertically infected babies and one HIV negative child from the formula feeding group died of gastroenteritis between six and nine months of age. All the HIV positive infants were started on antiretroviral therapy as per national protocol. One of the positive infants whose ART was delayed because of mother's reluctance to start (child's CD4 was 35%), died in hospital before 9 months (admitted with gastroenteritis; was on treatment for TB).

Table 28: End of study summary statistics

Parameters		Formula feeding (n=75)	Breastfeeding control (n=63)	Breastfeeding supplemented (n=66)
Mother status	**Alive (%)**	66 (88)	61 (96.8)	64 (97)
	Dead (%)	0 (0)	0 (0)	0 (0)
	Unknown (%)	9 (12)	2 (3.2)	2 (3)
Child status	**Alive (%)**	64 (85.3)	59 (93.7)	62 (93.9)
	Dead (%)	2 (2.7)	2 (3.2)	2 (3)
	Unknown (%)	9 (12)	2 (3.2)	2 (3)
HIV status child	**Negative (%)**	72 (96)	52 (82.5)	54 (81.8)
	Positive (%)	3 (4)	5 (7.9)	8 (12.1)
	Unknown	0 (0)	6 (9.5)	4 (6.1)
Mum started Antiretroviral therapy		3 (4)	2 (3.2)	6 (9.1)
Employed		28 (46.7)	14 (23.3)	18 (30)
New pregnancy		3 (4.1)	2 (3.2)	1 (1.5)
Vertical transmission	**Intrauterine/peripartum ***	3	5	6
	Postpartum/Breastfeeding transmission **	0	0	2

* Intrauterine/peripartum defined as positive HIV DNA PCR at ≤ 6 weeks
**Postpartum/breastfeeding transmission

3.11 *Population specific predictive equation for calculation of Lean body mass*

3.11.1 Using BIA Impedance values

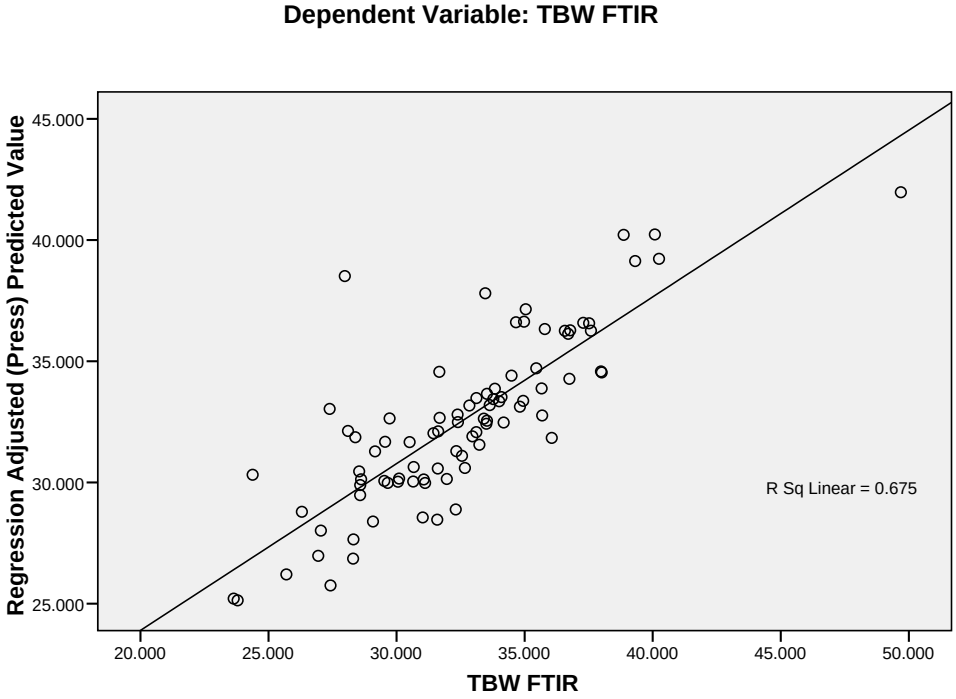
Using BIA impedance values and anthropometric measurements, it was found that the weight and height squared over impedance values at 50, 100 and 200 kHz were most significantly associated with the TBW values obtained through the FTIR (table 29a).

Table 29a: Pearson's coefficients for association with TBW (FTIR) for the various measurements

Measurement	Pearson's correlation coefficient	P value
Age (years)	0.308	0.003
Weight (W)	0.749	<0.001
Height (H)	0.306	<0.001
MUAC	0.644	<0.001
TSF	0.450	<0.001
BMI	0.607	<0.001
Height^2 (H^2)	0.305	<0.001
Impedance 5 kHz (R_5)	-0.622	<0.001
Impedance 50 kHz (R_{50})	-0.700	<0.001
Impedance 100 kHz (R_{100})	-0.696	<0.001
Impedance 200 kHz (R_{200})	-0.693	<0.001
H^2/ R_5	0.184	0.015
H^2/ R_{50}	0.810	<0.001
H^2/ R_{100}	0.810	<0.001
H^2/ R_{200}	0.808	<0.001

Through stepwise regression, the model with weight and H^2/ R_{200} was the model with the best fit with R^2 of 0.776 (figure 31).The validated equation for calculation of TBW using the BIA impedance was therefore: TBW= 0.153 W + (3322.783 H^2/ R_{200}) +5.931

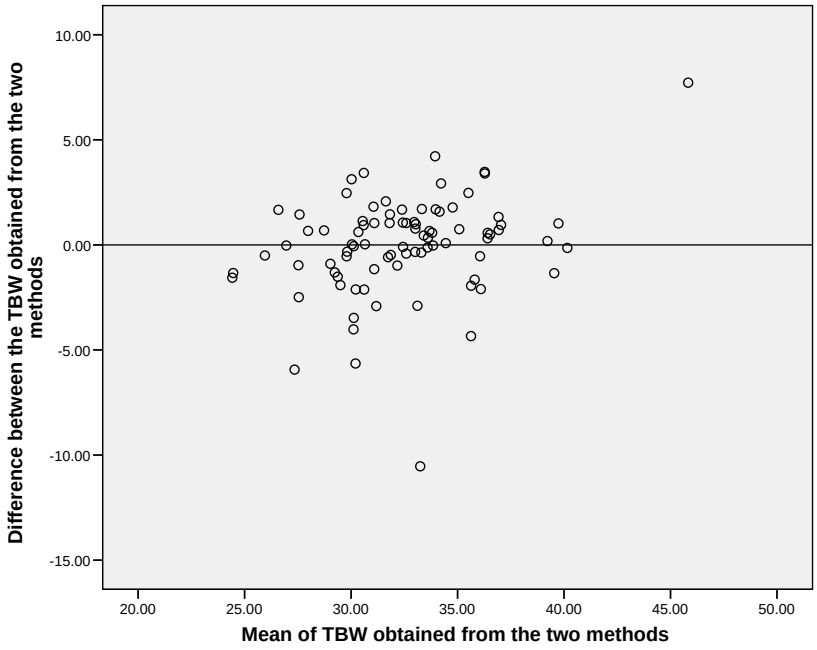
Figure 31: Scatterplot of TBW (FTIR) and the adjusted predicted value obtained from the equation using impedance values



The difference between and the mean of the adjusted predicted value and the TBW FTIR were computed and plotted in a Bland-Altman plot (figure 32).

There should be no correlation between the two values. The bias was calculated to be -0.001 with a 95% confidence interval 0f -0.509 and 0.507; p=0.996.

Figure 32: Bland Altman Plot to compare the two methods of obtaining the TBW (FTIR and BIA impedance values)



3.11.2 Using TBW obtained through the BIA

Using BIA impedance values and anthropometric measurements, it was found that the weight and height squared over impedance values at 50, 100 and 200 kHz were most significantly associated with the TBW values obtained through the FTIR (table 29b). However on doing the stepwise linear regression, the model using the TBW reading from the BIA had the best fit with a R2 of 0.761 (figure 33). The validated equation for estimating the TBW using the TBW obtained from the BIA is: TBW= 1.12 (TBW from BIA) – 3.586

Table 29b: Pearson's coefficients for association with TBW (BIA) for the various measurements

Measurement	Pearson's correlation coefficient	P value
Age	0.251	0.011
TBW(FTIR)	0.860	<0.001
Weight (W)	0.842	<0.001
Height (H)	0.338	<0.001
MUAC	0.737	<0.001
TSF	0.529	<0.001
BMI	0.699	<0.001
Height^2 (H^2)	0.338	<0.001
Impedance 5 kHz (R_5)	-0.775	<0.001
Impedance 50 kHz (R_{50})	-0.852	<0.001
Impedance 100 kHz (R_{100})	-0.845	<0.001
Impedance 200 kHz (R_{200})	-0.841	<0.001
H^2/ R_5	0.230	0.001
H^2/ R_{50}	0.958	<0.001
H^2/ R_{100}	0.956	<0.001
H^2/ R_{200}	0.954	<0.001

The difference between and the mean of the adjusted predicted value and the TBW FTIR were computed and plotted in a Bland-Altman plot (figure 34). The bias was calculated to be 0.006 with a 95% confidence interval between -0.478 and 0.49; p= 0.979.

Figure 33: Scatterplot of TBW (FTIR) and the adjusted predicted value obtained from the equation using the TBW (BIA)

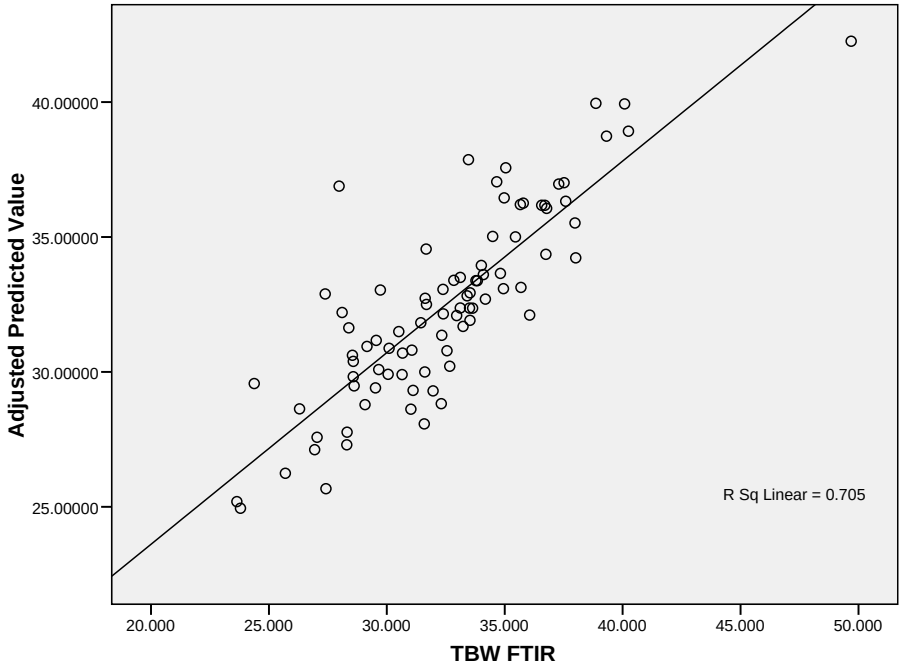
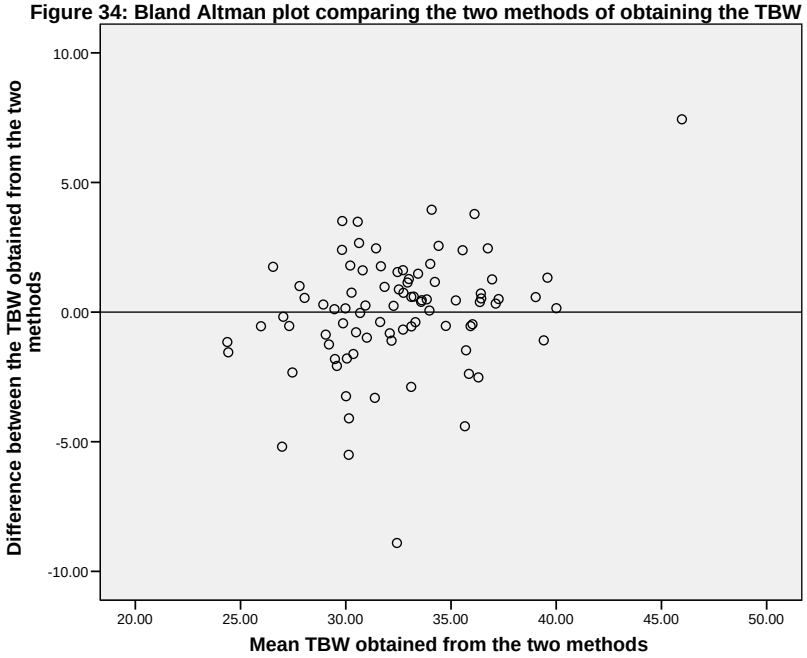


Figure 34: Bland Altman plot comparing the two methods of obtaining the TBW



3.11.3 Using anthropometric measures only (for the field)

Using stepwise linear regression, the model using weight and height had the best fit with a R^2 of 0.659 (figure 35).

The validated equation to calculate the TBW using weight and height obtained was:

TBW= 14.583(H in meters) +0.264(W in kg)-8.443

The bias for the equation is 0.002 with a 95% confidence interval between -0.628 and 0.633; p=0.994.

The Bland Altman plot was plotted using the difference between and mean of the adjusted predicted value and the TBW from the FTIR (figure 36).

Figure 35: Scatterplot between TBW(FTIR) and the adjusted predicted value obtained from the equation using weight and height

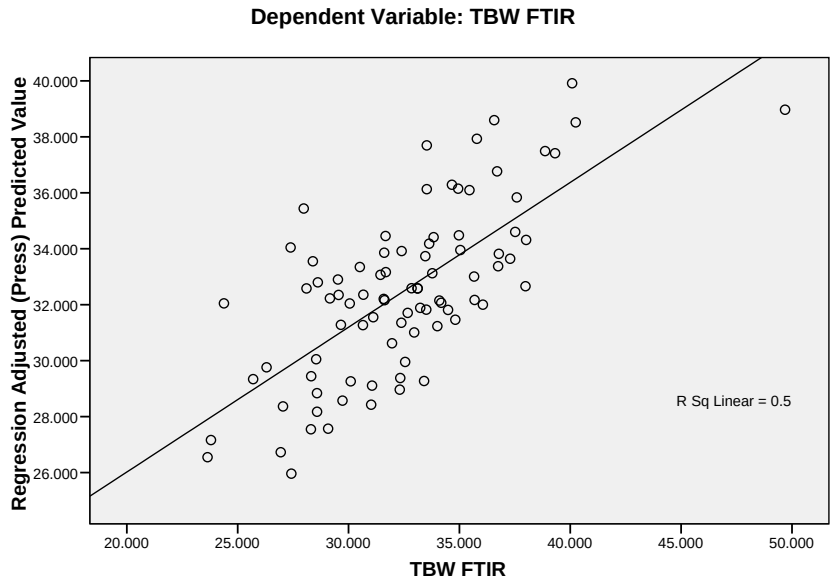
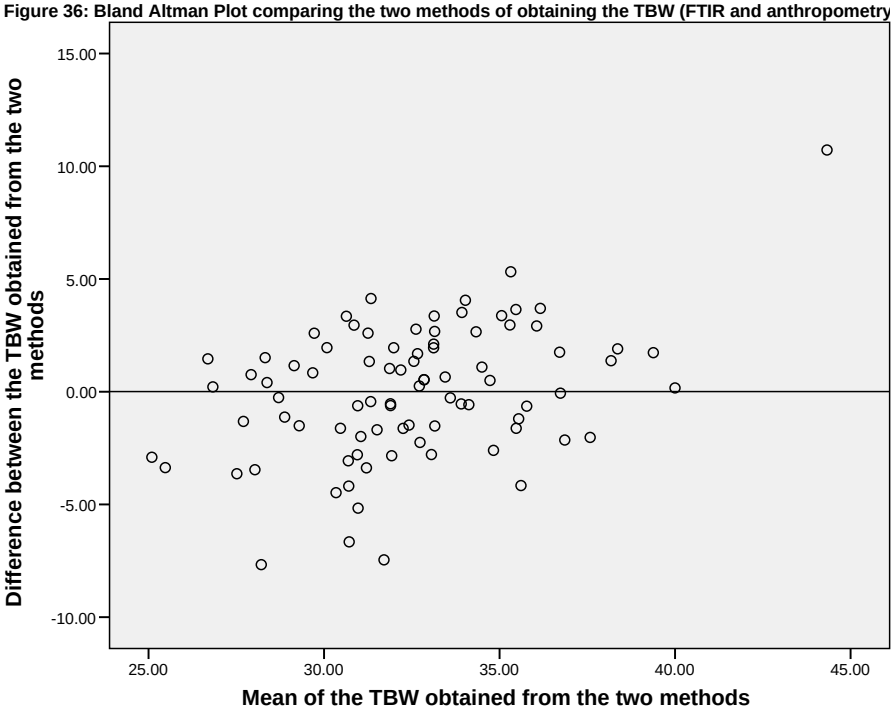


Figure 36: Bland Altman Plot comparing the two methods of obtaining the TBW (FTIR and anthropometry



Chapter 4

Discussion

Background socio-economic and nutritional data on the groups

As expected, randomisation was effective in producing two similar breastfeeding groups. However the formula feeding group which was self-selected was unusually similar to the breastfeeding control group. We expected to see a larger difference between the formula feeding and breast feeding group because women who select to formula feed normally satisfy AFASS criteria as per PMTCT guidelines and are usually socio-economically different. Although there were a higher percentage of formula feeding women having access to water, electricity and sanitation, the difference was not statistically significant. Similarly, although a higher percentage of formula feeders compared to breastfeeders had disclosed their HIV status, this difference was not statistically significant. This could be an indication that the infant feeding counselling that was supplied by the antenatal clinic was sub-optimal.

A large study conducted in KwaZulu-Natal showed that where women were well counseled the majority of women who selected to formula feed did in fact satisfy AFASS criteria and were different to the breastfeeding women in terms of the AFASS criteria (Bland et al, 2007; Rollins et al, 2008). Appropriate selection of feeding choice is very important in terms of HIV transmission as shown by Doherty et al (2007) in their prospective cohort study at three sites in South Africa. Infants, whose mothers chose formula inappropriately viz. not fulfilling the AFASS criteria, had an increased risk of vertical transmission of HIV and death (hazard ratio 3.63, 95% CI 1.48-8.89).

None of the women reported use of condoms as a form of contraception prior to enrolment, although use of injectable or oral contraception was good. This concurs with an earlier study in South Africa by Ngubane et al (2008) who found that "despite regular counselling from a study nurse and availability of condoms" very few women were using condoms. MacPhail et al (2007) also reported a very low use of dual contraception (7%) in South Africa. Low usage at our site was reportedly associated with partner's refusal to use condoms.

There were no mothers with BMI < 18.5 kg/m^2 (underweight as per WHO standards); 4 (2%) of our mothers had a BMI below 20 kg/m^2 at two weeks post delivery, this was similar to the findings of Papathakis et al (2006).

In our study, although the mean haemoglobin varied between 11.47 to 11.77 g/dL, 46% of the mothers had haemoglobin levels less than 11.5 g/dL. Low albumin levels (<32 g/L) were also observed in 45.6% of the mothers. These findings were also noted by Papathakis et al (2007) in her study in rural KwaZulu-Natal.

PMTCT prophylaxis and delivery

Most babies were delivered through normal vaginal delivery. Almost all (98%) of the mothers received PMTCT prophylaxis which is in keeping with a recent progress report by WHO/UNAIDS/UNICEF (2010) which estimated between 70-95% coverage. Amongst the infants, 94.6% of them received prophylaxis. There were 14 (6.9%) vertical transmissions detected at six weeks. There was a significant correlation noted with increased vertical transmission and low albumin (p=0.017); low birth weight (p=0.031); and a weaker correlation with mother's report of having had an episiotomy or a tear requiring suturing during delivery (p=0.075). These have been reported by previous studies (European

collaborative study, 1992; Pitt et al, 2000; Magder et al, 2005). We observed a significantly decreased HIV transmission risk in the group of mothers who received antenatal prophylaxis with combivir in addition to sdNVP compared to those mothers who received sdNVP only (2.9% vs. 13.4%, p = 0.019). This confirms a Cochrane review which showed that additional prophylaxis with one or 2 additional ARV drugs during the antepartum period significantly reduces HIV transmission (Volmink et al, 2007).

Our study showed that there was no association between haemoglobin levels and HIV transmission risk although previous studies have reported a strong correlation with low haemoglobin (Duri et al, 2010). This could be explained by the observation that our mean haemoglobin was higher than that reported in most studies. There was also no significant association with maternal CD4 counts (p=0.08). This could perhaps be because all our mothers had counts above 200 cells/µLand 65% had CD4 counts above 350 cells/µL which was not the case in previous studies (Mofenson et al, 1999). No viral loads were done at baseline; therefore these associations were not assessed.

Changes in haematological and biochemical parameters in mothers over six months

Haemoglobin levels were low in 34% of the mothers at six months; this was associated with a low haematocrit in 55-60% of the mothers; and a low MCV in 9% of the mothers suggesting an underlying iron deficiency. MCV was high in 19% of the mothers at baseline returning to normal at six months; this is in keeping with the normal physiological changes in MCV during pregnancy when the MCV increases as the erythrocytes become more spherical due to increased diameter and an increased thickness. Since almost half of the group received antenatal prophylaxis with combivir (zidovudine and 3TC) there could be an associated effect of zidovudine on both anaemia and a raised MCV which is usually reversible (Thorne and

Newell, 2007). The MCV returned to normal by six months post delivery. These findings confirm the findings of Papathakis et al (2007) who also found low haemoglobin in 33% of her HIV positive lactating mothers; we however found that the low haemoglobin was irrespective of the type of feeding mode.

There were no mothers with folate deficiency, which was quite different from the findings of Papathakis et al (2007). Perhaps this can be accounted for by good antenatal adherence to the routine daily folate supplementation (supplied as routine care) as well as increased availability of fortified foods especially maize meal (DOH, 2003).

More than half (67.6%) of the mothers had low zinc levels; similar to the findings of Paphathakis et al (2007). In spite of all three groups having an increase in zinc levels, 50.5% still had low levels at six months. The increase in zinc levels in the breastfeeding groups was significant however the increase in the formula feeding group was not significant possibly due to the fact that their mean baseline level was higher (and normal) compared to the breastfeeding groups (64.32 µg/ml vs. 59.18 and 57.5 µg/ml). There is concern that half of the women postpartum had low zinc levels at six months as zinc is an anti-oxidant and plays an essential role in both humoral and cellular immunity (Shankar and Prasad, 1998; Sazawal et al, 1997; Fraker et al, 2000). Zinc has also been shown to be important in maintaining the integrity of the gut mucosa (Wapnir, 2000; Roy, 1992) which is of significance in the context of breastfeeding and vertical transmission. As we had only two late postnatal transmissions that could be attributed to breastfeeding, we were not able to investigate any association between breastfeeding transmission and low zinc levels.

B12 deficiency was not common (2%), this finding was quite different from Papathakis et al (2007), who found almost 60% of the population was B12 deficient or had marginal B12 levels. The fact that our population was more urban, may explain why this deficiency was not noticed to the same extent.

Changes in body composition and anthropometric measurements in mothers over six months

There was a significant change within each group for all the measurements over six months; the change was however similar. All three groups lost weight between two weeks post delivery and six months as is expected normally post delivery. There was a significant change in lean body mass and fat mass; all mothers lost lean body mass and gained fat mass; percentage fat mass; MUAC; and TSF measurements. These findings are different to Papathakis et al (2006) who found that the HIV positive lactating mothers had a decrease in TSF measurements and no significant changes in lean body mass and fat mass. Loss of lean body mass has been well documented in HIV infection (Kotler et al, 1989; Grinspoon et al, 1998).

Various factors are associated with body composition changes during lactation and therefore body composition changes during lactation are highly variable (Butte and Hopkinson, 1998). One of these factors is low protein intake; the dietary intake in our group as a whole showed low protein intake which could account for the decrease in lean body mass (Motil et al, 1998). Furthermore it has been shown in a longitudinal study in adolescents (Bonny et al, 2009) that use of medroxyprogesterone was associated with loss in lean body mass and an increase in fat mass; of note 95% of the women in our group were on medroxyprogesterone contraceptives.

Effect of breastfeeding on nutritional status and health of mothers

There were no significant differences between the formula feeding and breast feeding mothers at 14 weeks or six months post delivery for any of the anthropometric or body composition measures. As expected there was a drop in weight and BMI during the first 6 months of breastfeeding however the drop (0.43 kg) was not more than expected (Papathakis et al, 2006) and in fact was less than that experienced by the formula feeding mothers (1.14kg). We decided to probe further into the proposed ill-effect of weight loss by examining a more precise indicator viz. LBM. We found that even when examining loss of LBM over the 9 month period there was no difference between the breastfeeding and formula feeding women. In fact the loss of LBM was slightly higher in the formula feeding women; although not significant. On assessing further, for associations between BMI and mother's CD4 counts, we observed that being older and having a higher CD4 count was significantly associated with higher fat mass and subcutaneous fat measurements.

There was also no evidence of a negative effect of breastfeeding on any of the haematological or micronutrient variables examined. In fact the breastfeeding group tended to have higher haemoglobin and haematocrit levels than the formula feeding mothers, although the difference was not significant.

The formula feeding group compared to the breastfeeding group had more women progressing to WHO stage 3 or higher (4 vs. 0). Again this is reassuring as it provides further evidence that lactation is not harmful for HIV infected mothers as was previously suggested by Nduati et al (2001).

Significantly fewer breastfeeding women relative to formula feeding women experienced depression as evidenced by having a SRQ score of ≥ 8 (p=0.043). A few other studies assessing factors associated with post partum depression found that formula feeding was one of the factors associated with higher odds of postpartum depression (Lane et al, 1997; Mezzacappa and Endicott, 2007; Dennis and McQueen, 2009). The exact mechanisms for this are not clear but it may be related to the hormonal benefits of oxytocin (Lee et al, 2008). The higher depression scores in formula feeding women could also be associated with the considerable burden/guilt that mothers carry when they are expected to make a choice against a behavior like breastfeeding that is considered a normative behavior in their culture. In the light of this observation the new 2010 KwaZulu-Natal PMTCT guidelines (KZN DOH, 2010) which are based on the WHO recommendations (2010a) are very encouraging as they take away the burden of choice and guilt from the mother and instead mothers are presented with one option (as they are with all other public health interventions). Women should therefore feel more confident that policy makers have considered evidence and have made a value judgement on which intervention (breastfeeding with infant prophylaxis or formula feeding) provides the best health and survival outcome for their infant.

Effect of breastfeeding on child growth, development and health

Baseline anthropometric measures were similar in all groups. Infants in the breastfeeding control group did significantly better in terms of all growth parameters by 14 weeks of age viz. weight gain, z scores for weight for age, BMI for age, weight for length, MUAC for age and TSF for age. However by six months, though the mean measurements were higher in the breastfeeding group, only the mean BMI remained significantly higher (p=0.045) which could be explained by the fact that 26% of the breastfeeding control group had discontinued

breastfeeding by four months of age. Our results are in keeping with those of other studies with formula fed and breastfed HIV exposed infants (Arpadi et al, 2009; Patel et al, 2010). Truncated Denver development score was significantly better (p=0.03) in the breastfed infants at 14 weeks compared to the formula fed infants and significantly more (p=0.047) breastfed infants had achieved age appropriate milestones by that time; this difference was no longer significant at six and nine months which could be due to early cessation of breastfeeding by some of the mothers as explained above. These benefits of breastfeeding have been well documented (WHO, 1999).

Breastfeeding was significantly associated with a 69% lower incidence of diarrhoea at 3 months (p=0.006). There was also a significantly higher incidence of hospital admissions in the formula-feeding group (7 vs. 0; p=0.014); as well as more visits with infants not having attained their age appropriate milestones (5 vs. 0; p=0.047). These however became not significantly different at six months and later. Once again the benefits of breastfeeding in decreasing the risk; as well as hospitalization for diarrhoea has been well documented (Creek, 2010; Bahl et al, 2005).

When we examined the HIV infected infants as a separate group, we were not able to show that the breastfed infants had a better growth and health outcome than the formula fed infants as has been reported by others (Kuhn and Aldrovandi, 2010a; Taha et al, 2010) presumably because of the very small number of infected infants (n=8). However we were surprisingly able to show at 3 months of age a significantly higher number of breastfed infected infants had achieved age appropriate milestones compared to the formula fed.

Adherence to nutritional supplement

It had been assumed that the supplement was acceptable in our community as it was being used by the provincial heath department in hospitals and clinics. However we found that a considerable majority of the mothers counselling to the supplemented group had variable adherence to the supplement. The duration of taking it appropriately was significantly associated with the mother's assessment of the food paste; if they had no particular dislike to the paste, they took it for longer (Median 4.5 months); whereas if they thought it had no particular advantages or thought it to be too rich or sweet in taste they took it for a median of one and 2.5 months respectively.

Dietary assessment

The diet of the mothers was assessed to control for the dietary intake when analyzing for the effect of the nutritional supplement. Around 32% of our mothers reported food insecurity, some of them having eaten very little/ nothing in the last 24 hours. The mean energy intake was within normal levels at 14 weeks for a person with limited/no physical exercise however the protein intake was very low; (less than the RDA 71g/day) for lactating women. The composition of the diet became increasingly lower in energy, protein and micronutrient content by six to nine months which re-emphasizes the need for increasing empowerment of women by job creation and providing sustainable means to improve food security.

Effect of nutritional supplementation on nutritional status and health of mothers

The two breastfeeding groups were similar in terms of changes in anthropometric measurements and body composition changes, however it was noted that although both groups lost lean body mass, there was a lower loss in the supplemented group compared to the control group; p value was not significant. This effect was further investigated in a sub-group

analysis, where the groups where further divided into normal to low (<25 kg/m^2) and high to obese (≥25 kg/m^2) categories; this was done to assess if the mothers on whom lactation would be an extra metabolic demand i.e. the mothers with a low BMI were not being more compromised. It was noted that the supplemented mothers with a low BMI actually lost a significantly lower amount of LBM compared to the control mothers (1.32kg vs. 3.17 kg; p=0.026). Even when controlling for baseline age, CD4 and duration of breastfeeding, this effect remained. The nutritional supplement to the group of lactating mothers with low BMI was therefore effective in preventing some loss of lean body mass presumably by providing additional protein to compensate for low dietary protein intake. There have been no published studies to our knowledge assessing the impact of nutritional supplementation on changes in lean body mass of breastfeeding mothers. Prentice et al (1980) observed increased subcutaneous fat and increased weights in their study of nutritional supplementation of breastfeeding mothers; however no body composition measurements were done.

Our analyses revealed an independent effect of breastfeeding duration. In the group of mothers with high BMI, mothers who breastfed for a longer duration gained more weight and had higher fat mass and fat percentage than the mothers who ceased breastfeeding by 3.5 months. In the mothers with a low BMI, there was no difference for any of the body composition measures between the mothers who breastfed for longer compared to the ones who stopped early. In a study of postpartum weight changes in Zambian HIV infected mothers, Murnane et al (2010) found similar effects and suggested that, "weight change associated with longer breastfeeding may be metabolically regulated so that women with low BMI and at risk of wasting are protected from excess weight loss".

The nutritional supplement had no impact on any of the haematological or micronutrient parameters we investigated even in the women with low BMI. On assessing within the

supplemented group for any differences per duration of breastfeeding/supplementation; there were no significant differences within the subgroups for any of the laboratory parameters except for CD4 count which was significantly higher in the mothers who took the supplement for longer (22 cells/μL) than the ones who stopped before 3.5 months. However on further analysis, this impact was shown to be due to breastfeeding duration and not the supplement. The CD4 count in the group with longer duration of breastfeeding was significantly higher by 74 cells/μL (p=0.040). This could be due to reverse causality as mothers who may have felt symptomatic, may have decided to stop breastfeeding earlier.

No other published studies have reported impact of nutritional interventions in postpartum women. However, our results are confirmed by findings in a Cochrane review (Mahlungulu et al, 2007) of nutritional interventions in HIV infected men and non postpartum women which similarly found no impact on these parameters.

There were no differences between the groups for incidence rates of any of the opportunistic infections on cross-sectional analysis. However when we studied the supplemented group for intra group differences for the duration of breastfeeding/supplementation; there was a significantly lower incidence of LRTI and PID; and a higher Karnofsky score amongst the mothers who took the supplement for longer. When examining this association further for duration of breastfeeding by using a generalized linear model with Poisson distribution and log function (controlling for baseline CD4 count and BMI). Supplementation was significantly associated with a lower incidence of genital ulcers. This is difficult to explain since none of the other morbidities were influenced and this therefore may be a chance finding. Longer duration of breastfeeding was significantly associated with less breast pathology and a lower incidence of PID and genital ulcers. The reason for less breast

pathology could be explained by reverse causality. The lower incidence of PID and genital ulcers could perhaps be explained by cultural tendency to abstain from sex during the first six months of breastfeeding or as explained earlier there may have been some effect of reverse causality.

Effect of maternal nutritional supplementation on nutritional status and health of the infants

In the initial analysis the control group appeared to have significantly higher MUAC, TSF and BMI measurements. We then assessed the infant's growth as per duration of maternal supplementation within the supplemented group and observed significantly higher measurements and z scores with a longer duration of supplementation/breastfeeding. We then proceeded to do a longitudinal analysis using generalized estimating equations adjusting for maternal CD4 count and BMI and duration of breastfeeding, there were no significant differences between the two groups except for the TSF/age z score which was lower in the supplemented group than the control groups at any time. There was a significant association of longer breastfeeding (irrespective of supplementation) with higher measurements and z scores for all parameters except the z score for HC/age and weight/length. All the mothers who breastfed up to six months were exclusively breastfeeding, there was a very low incidence of mixed feeding amongst our mothers; these were the mothers who eventually stopped breastfeeding by 3.5 months. The positive association of six months exclusive breastfeeding on child growth has been shown in a secondary analysis by Vesel et al (2010). The infants who breastfed for longer had a significantly higher likelihood of achieving their age appropriate milestones. This has been reported earlier by Horwood and Fergusson (1998) who observed that children who had been breastfed for longer had better cognitive and educational outcomes at a later stage. It also confirms the findings in a review by WHO (1999).

Nutritional supplementation to the mothers had no impact on opportunistic infections in their infants. On using the generalized linear model with Poisson distribution and log function however; increased duration of feeding irrespective of group significantly reduced the risk of skin sepsis, LRTI, hospital admissions and death. This positive effect of longer duration of breastfeeding has been reported recently in a Zambian study (Kuhn et al, 2010b).

On assessing the very small group of 13 infected infants in the two breastfeeding groups, there were no significant differences between those in the supplemented and control groups in terms of growth and development. Longer duration of breastfeeding was observed to have a significantly positive impact on all growth parameters except for the length/age and HC/age z scores.

There were no significant differences between the incidences of opportunistic infections in both groups.

Effect of nutritional supplementation on breastmilk composition

There were no significant differences between the two groups with regards to both macronutrient content and the micronutrients investigated by us. The protein, fat and energy content of breastmilk were similar to the normal standards. By six months, zinc levels fell in the breastmilk; similar results have been reported previously in the literature (Brown et al, 2009). The impact of supplementation is very likely to be affected by factors such as: timing of supplementation; underlying nutritional and health status; dietary intake; and dose of the supplement. It has been shown previously by Chierici et al (1999) that zinc supplementation during lactation does not have an effect on zinc levels in the breastmilk; however in a study of

undernourished Senegalese women (Cisse et al, 2002) observed that supplementation during pregnancy made a significantly increased the breastmilk content of zinc.

The mothers had a high breastmilk retinol level (presumably due to the 200,000 IU given postpartum to all our mothers) at two weeks post delivery which had decreased by six months but was still within the normal reported retinol levels in both groups. Oliveira-Menegozzo et al (2010) in their review of all the available literature on postpartum high dose vitamin A observed that the increase in retinol levels of breastmilk is not sustained beyond three months. Our nutritional supplement which contained 75% of the RDA for vitamin A did not seem to have made any impact on the vitamin A levels in breastmilk.

Cervical screening of mothers

All mothers were offered cervical screening during their six month follow-up visit. However, only 42.6% agreed to have a screen at the visit. 24 (27.6%) mothers had a cervical lesion suggestive of a human papilloma virus infection (HPV); 20 had a low grade squamous intraepithelial lesion and four had a high grade lesion requiring further investigation and colposcopy.

According to the WHO/ICO (Information Centre on HPV and Cervical Cancer (HPV Information Centre), 2010); cervical cancer is the second most prevalent cancer amongst women in South Africa. The burden is particularly high amongst women aged between 15 and 44 years of age; but the coverage of cervical screening is very poor around 13.6% (WHO/ICO, 2010). Batra et al (2010) have also highlighted the low prevalence of cervical screening in Cape Town. This is an important clinical problem where morbidity and mortality can be reduced by regular screening (Kawonga and Fonn, 2008). Mothers are often rushing to work or have their children with them when they come to the clinic and are not willing to wait

to have the procedure. Our study highlighted this problem of low levels of cervical screening and calls for concerted efforts to facilitate increased cervical screening. There have been some trials of rapid screening (by visual inspection with acetic acid aided by digital cervicography and referring the subjects with suspicious lesions for further investigation) which could be an easier alternative for the mothers as well as the health care staff thereby increasing the uptake of screening (Parham et al, 2010).

Infant feeding practice

As mentioned earlier, only 38.7% of the mothers who chose to formula feed actually fulfilled the AFASS criteria. There is still a need to increase the knowledge on breastfeeding practices amongst health care workers in the hospitals where most of our mothers delivered as only 34% of their infants were put to the breast within the first hour of delivery. This of course could also be due to the disempowerment of our mothers when faced with more educated health care workers and a need for them to conform to whatever is being advised or told to them rather than taking part in decision making. A majority of the mothers seen by us informed us that they were often told by the nurses to formula feed despite the mothers having indicated her choice prior to delivery and this being recorded in her file.

It was very encouraging that 57.4% of the mothers reported that they were still exclusively breastfeeding at six months considering the very low levels of exclusive breastfeeding reported in the South African Demographic Health Survey (2003). This highlights that it is possible to improve exclusive breastfeeding rates with supportive counselling during clinic visits. A previous study in rural KwaZulu-Natal with frequent counselling also reported higher levels of exclusive breastfeeding (Coovadia et al, 2007).

Better access to basic amenities was significantly associated with longer duration of breastfeeding. This is a complex indicator and could be a proxy for empowerment of women which enables adherence to behavioural practices.

Status of mothers and infants at end of follow-up

Six of the infants died; of which 5 were HIV-infected and 1 was HIV negative. The HIV negative death was in the formula feeding group and was secondary to gastroenteritis. The other five deaths were all secondary to gastroenteritis; one formula fed infant and four breastfed infants. All the deaths in the breastfed infants occurred within a few months of breastfeeding cessation (three stopped before three months and one at six months). The early cessation of breastfeeding was found to be significantly associated with an increased risk of death (p=0.045). Therefore our findings endorse the importance of the revised WHO (2009b) recommendations which encourage breastfeeding for 12 months (in the presence of ARV prophylaxis) because they have taken into account not only HIV infection but also child survival. Our National Government endorsed the guidelines which were officially implemented on the 1st of February 2010 (DOH, 2010).

Predictive equations for body composition using BIA and anthropometric measures

Generic BIA equations have been in use in South Africa to calculate lean body mass; however these are often not representative of the population being studied, as they are generated from western populations. It is therefore important that we have South African population specific predictive equations.

We therefore used the values for TBW obtained from the deuterium enrichment method (gold standard) and by performing stepwise linear regressions, were able to form three predictive

equations. These were firstly, one for use in the field (using weight and height only); secondly one for use with BIA impedance values; and thirdly one for use with machines that generate a TBW value. These equations can be used in the female Zulu population.

Limitations

The nutritional supplement used was variable in acceptability and resulted in lower than expected adherence to six months of supplementation. This could have influenced our findings; although we took this into account by controlling for duration of supplementation. The other limitation of the study was that the supplementation was not given as a meal replacement but as a supplement at a dose of 50g/day; which only covered a fraction of the macronutrient requirements; however we did compare the dietary intake of the two groups of breastfeeding mothers which as reported was similar to ensure that we could study the impact of the supplement without bias. In assessing micronutrient levels in particular zinc and vitamin A, we were unable to comment whether the lower levels were associated with deficiencies because we did not concurrently measure any of the acute phase proteins (due to insufficient funding). Samples have however been stored and kept aside in the event funds become available in the future.

The final limitation of the study was that perhaps we were too ambitious when we calculated the sample size, hypothesizing a difference in lean body mass of 4 kg between the supplemented and non-supplemented groups. We decided to choose an effect that would be substantial enough to justify the cost and logistics of introducing the type of supplement we were using. However this therefore meant that we did not have power to detect differences in lean body mass of less than 4 kg and we also did not have sufficient power to detect any effect of the supplement on CD4 count and other parameters.

Chapter 5

Conclusions and Recommendations

Breastfeeding was not associated with any adverse effect on an HIV positive mother nutritional status and in fact appeared to be protective to the mother's mental health. Longer duration of breastfeeding (>3.5 months) was significantly associated with an increase in CD4 counts in the mothers.

There was a positive impact on child growth and development and lower incidence of diarrhoeal disease seen amongst the breastfed infants. Longer duration of breastfeeding (>3.5 months) was protective to the child both in terms of clinical events (skin sepsis, LRTI, hospitalization, and death); growth and development of the child.

The nutritional supplement had limited impact namely protecting loss of lean body mass in a select group of mothers with low BMI.

We have successfully developed South African Zulu population specific prediction equations which can be used for research and clinical monitoring of important prognostic anthropometric indicators.

Dual prophylaxis was significantly better than sd NVP in reducing vertical transmission.

The low uptake of cervical screening by the mothers is worrying, in view of the high risk of human papilloma virus infections in this population and the possibility of reducing morbidity and mortality by timely screening.

Recommendations

In view of the importance of breastfeeding and the fact that we have shown that it is possible to maintain good exclusive breastfeeding rates with continued support by well trained feeding counselors who empathise with the mothers and their problems; we recommend continued efforts to train and empower lay counselors. Well trained empowered counselors can in turn empower the mothers to be able to make wise decisions and be able to persevere with their feeding choice despite being given mixed information by other health care workers. There is also a need to increase the numbers of baby friendly hospitals and clinics and educate our health care workers well, so that they understand the reasons for promoting breastfeeding which in turn will remove any feelings of guilt that arise from giving disparate advice.

There is therefore a need to ensure proper understanding of the AFASS criteria by the health care workers as well as the mothers so that appropriate feeding choices can be made.

Since nutritional supplementation had no impact on the group of mothers as a whole and only a limited effect in mothers with low BMI, we would recommend that nutritional supplements should only be provided in a well thought out targeted approach. Nutritional supplement interventions often do not make an impact due to nonadherence. Nonadherence can be due to exchanging the product; selling it; sharing it; or subjects with any socially stigmatizing disease often do not want to be identified and therefore try to hide the supplement or even throw it away. This was observed by Doherty et al (2006) when they conducted a qualitative interview study amongst HIV positive mothers with regards to their infant feeding practices.

Although there is a place for food parcels in emergency situation such as environmental crises, civil wars etc, their use in routine practice especially in today's environment of global economic instability is not sustainable and is disempowering. There is a need to put people in control of their own health. One of the solutions could be to include simple and easy to follow nutrition guidelines during health education in primary health care clinics and antenatal clinics. Davies et al (2009) have successfully shown in their "breaking the cycle project" that by selecting peers in a targeted community and training them in nutrition education as well as using culturally acceptable recipes helped them to engage with the community. This led to a significant change in dietary habits. Frison et al (2006) reiterate the importance to include a "holistic food based approach" which could increase the use of nutritionally valuable traditional food which can be grown easily, be sustainable and more empowering.

One of our findings was the poor quality of diet in terms of low protein content relative to the carbohydrate and fat content; Vorster et al (2004) have shown in their population based study that the intake of polyunsaturated fats albeit at a low level seemed to have a negative impact on the liver enzymes. This again re-emphasizes the urgency for good dietary counselling which could perhaps be peer-driven to be initiated while the subjects are still relatively well, so that their liver function be optimal (most antiretroviral are metabolized by the liver), by the time they need to go on to ART. Another recommendation related to dietary intake is to encourage mothers to exercise in the post partum period, which would help to increase the lean body mass as well as improve their cardio-respiratory fitness (Amorim et al, 2007). We are of course not recommending dietary restriction but advising healthy eating by diet modification and lifestyle changes. This is in view of the well documented rise in cardiovascular and metabolic related morbidity and mortality amongst the HIV infected population (Mutimura et al, 2008).

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Appendix 1

Nutritional Analysis									
Sibusiso Ready Food Supplement									
Nutrients		Per 100 g	Per 50g	% RDA * per 50g					
			Daily Serving	Daily Serving					
Energy	- kj	2352	1176						
Protein	- g	16	8						
Carbohydrate	- g	48	24						
Fat	- g	35	17.5						
Fiber	- g	1.9	0.95						
Vitamin A	- mcg RE	1200	600	75					
Vitamin D	- mcg	10	5	83					
Vitamin E	- mg	30	15	100					
Vitamin K	- mcg	50	25						
Vitamin C	- mg	150	75	100					
Vitamin B1	- mg	2.2	1.1	79					
Vitamin B2	- mg	2.2	1.1	69					
Vitamin B3	- mg	20	10	56					
Vitamin B6	- mg	2.6	1.3	50					
Folic Acid	- mcg	600	300	75					
Vitamin B12	- mcg	3.6	1.8	60					
Biotin	- mcg	60	30	100					
Pantothenic Acid	- mg	8	4	80					
Calcium	- mg	2000	1000	91					
Phosphorus	- mg	1400	700	80					
Iron	- mg	28	14	100					
Magnesium	- mg	160	80	23					
Zinc	- mg	10	5	33					
Iodine	- mcg	300	150	100					
Selenium	- mcg	110	55	100					
Sodium	- mg	<580	<290						
Potassium	- mg	1150	595						
Copper	- mcg	1400	700						
* Percentage Recommended Dietary Allowance Adults and Children older than 10 years									
INGREDIENTSSugar, peanut paste, soya milk powder, vegetable oil, soya protein, vitamins									
and minerals, vanilla flavour									
Suitable for vegetarians									
Not suitable for those with peanut or soya allergies									
This product is made out of products that are naturally lactose free									
RECOMMENDED SERVING									
9 months to 2 years – one to two heaped teaspoons a day									
2 years to 10 years – two to four heaped teaspoons a day									
Children older than 10 years and adults – four to six heaped teaspoons a day									
NOTE:Because this is a food supplement, intake should be dependant on the individual's									
nutritional needs									

Appendix 2

UNIVERSITY OF
KWAZULU-NATAL

Research Office
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06 May 2006

Dr G Kindra
Department of Paediatrics
Nelson R Mandela School of Medicine

Dear Dr Kindra

PROTOCOL: Effect of nutritional supplements on lean muscle mass, general health and disease progression of HIV positive lactating mothers and the ensuing effects on their infants including the impact of feeding mode on infant immune response to infection. G Kindra, Paediatrics. Ref: H081/05

Thank you for your response dated 24 April 2006 to queries dated 21 April 2006.

A sub-committee of the Biomedical Research Ethics Committee has considered your responses to the queries raised. The conditions have now been met and the study is given full ethics approval and may begin as at today's date 06 May 2006. Your revision to the above title has also been noted and approved.

This approval is valid for one year from **06 May 2006**. To ensure continuous approval, an application for recertification should be submitted a couple of months before the expiry date. In addition, when consent is a requirement, the consent process will need to be repeated annually.

I take this opportunity to wish you everything of the best with your study. Please send the Biomedical Research Ethics Committee a copy of your report once completed.

Yours sincerely

DR J MOODLEY
Chair: Biomedical Research Ethics Committee

Appendix 3

INFORMATION DOCUMENT

Title of study: Effect of nutritional supplements on lean muscle mass, general health and disease progression of HIV positive lactating mothers and the ensuing effects on their infants including the impact of feeding mode on disease progression of the infected infants

Short Title: *Influence of nutrition on health status of mothers and children*

Sawubona!

PURPOSE OF STUDY:
We are from the Department of Paediatrics and Child health, University of KwazuluNatal and are doing a study, which is a method that helps to answer questions regarding any problem that is affecting a population.
Therefore we are asking for your cooperation and assistance in helping us to answer some of the questions.

As you know as part of the routine antenatal clinic follow-up, HIV positive mothers are counseled about breastfeeding or formula feeding their infants, and mothers are asked to choose the best option for their babies in their particular situation.
We would like to determine how your health is affected according to whether you breastfeed or formula feed. This will involve taking bloods and breastmilk (only if you are breastfeeding), to measure the amount of vitamins, proteins and cells in the blood and breastmilk We will also measure your weight, height, skin fold and we will do a special test which involves swallowing some water which has a special chemical marker to be able to work out how much of your body weight is muscle. This water with special marker is completely safe and endorsed by the World Health Organization and has been used in many studies before, to test what is happening to the muscle in the body. We will also ask you for a saliva sample before you drink this dose of water and 3 hours after you drink the water. The day we do this test we shall ask you to not to eat 1 hour before drinking the water and for 3 hours after, following this test you will be supplied with lunch at the clinic. These tests will be done at 1/52 after delivery, 14 weeks after delivery, 6 months and 9 months.
In addition, for those women who are breastfeeding we want to be able to work out if it helps the mother's health if she receives a special food paste containing multivitamins. To work this out we need to do a study where we compare mothers who receive this paste compared to those mothers who do not. The only way to test this is for us to allocate mothers in a random way into these 2 groups. Mothers will be asked to choose a card which will say on it which group they will be assigned to. One group of mothers will receive for 6 months a monthly supply of special food paste for themselves to eat or a monthly supply of tea and cleaning products of equal monetary value.
Therefore mothers will be in one of 3 groups – the cost of the items supplied will be the same in each group:
Formula feeders who will receive 6 months formula for their babies and multivitamins for themselves
Breastfeeders receiving food paste and multivitamins for themselves
Breastfeeders receiving tea and cleaning supplies and multivitamin tablets

If you are willing to allow your child and yourself to be a part of this study, we shall be very grateful. If at any time, you wish to withdraw yourself and your child from the study, please let us know, it will not be reflect upon the care that will be provided to you by the routine clinic staff.

Study visits:

If you agree to participate in the study these are the visits you and your baby will be asked to attend the clinic for clinical examination which will involve weight, height, and arm measurements and examination of any signs of infection or sickness at:

1 week after delivery, monthly thereafter till 6 months.
At 6 months the supplement will be stopped and we shall see you three monthly thereafter depending on your and your baby's health.
The WHO recommends that at 6 weeks, your child be started on Cotrimoxazole, a medication given to all babies who are born to HIV-1 positive mothers in order to protect them from a serious type of pneumonia.

Procedures:

Bloods (8ml about 1,5 teaspoons) will be taken from the mother at the 2 week, 14 weeks, 6 month and 12 month visits.
Blood from child: heel prick at birth, (about one teaspoon) will be taken from the baby at 2 weeks, 6 weeks, 6 months, and 12 months.
Breastmilk will be collected from breastfeeding mothers at 2 weeks and 6 months.
Saliva will be collected from mothers at 2weeks, 14 weeks, 6 months and 9 months.

HIV tests:
At 6 weeks, the baby's HIV test will be done as required by the PMTCT program.
If you are breast-feeding and your baby is negative, we will re test your baby at 9/12 or 6/52 after you stop breast-feeding.
All the babies who test positive will have CD4 counts done. This is a test, which helps to guide us when your child needs to start treatment. If your child needs treatment either because of his/her low CD4 count or because he/she is ill or not growing well, he/she will get treatment from either, the MTCT plus program or our roll out clinic. We shall be doing CD4 counts every 6 months on all the positive babies and all the mothers, so that we can refer you timeously for treatment. This will be done regardless of whether you decide to continue with our study or not. We will be working in conjunction with the antiretroviral therapy clinic/MTCT plus program; therefore there will not be any delay in the management of you or your child.
We shall be updating you of the results as soon as they are available.

We will provide outpatient treatment you and your baby may need for any problems we determine at the visit and if necessary we will refer you to the appropriate facility.

Risks:
There are no known risks associated with this study. There may be a slight swelling and redness at the site of venepuncture, which will resolve. Every care shall be taken to 138tabiliz pain to the child at the time of venepuncture.

Benefits:
The advantages of being on this study are that there will be access to the same clinician at all times and regular follow up and timely referral if you or your child needs therapy.

This study will potentially benefit HIV-positive women as it will give us information on what feeding methods are the best for the health of the mother and child. As the results of the study will not be available for about 24 months you yourself will unfortunately not directly benefit from this study.

Confidentiality:
Every effort will be made to keep all the information given to us confidential, the names will be known to the study clinician only and every child will be allocated a study number by which they will be known. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law.
Specimen containers will be 138tabiliz with a study number and not your name. All of the research data will be securely stored at the University of KwaZulu-Natal. Publications resulting from this study will not identify you or your infant by name or any other identifying information.

Costs/Compensation:
There will be no cost for you to participate in this study. We will provide you with a snack and drink on your clinic visit day and you will be given R30 to reimburse for your transport costs and inconvenience for coming to the clinic.

Rights to refuse or withdraw:
You may choose not to participate in this study. There will not be any force applied upon you to take part in this study, whatever your decision maybe, it will not reflect upon the care that your child would have routinely received; withdrawal from the study is possible with informing the study clinician.
The investigators may withdraw subjects at their discretion.

Principal investigators' disclosures of personal and financial interests in the research study and sponsor
The investigators and co-investigators have no financial interest in this research and will not benefit monetarily from this investigation.

Please note this study has been approved by the Ethics Committee – approval no: H081/05

If any problems arise /or you need to query any problem with your child or otherwise, you must contact the study clinician:

Dr Gurpreet Kindra 0829631921/3603693

For reporting of complaints / problems:

If you have any concerns please contact the University of KwaZulu-Natal, Research Ethics Committee on 031-2604410.

Chairperson: Prof J Moodley – tel: 031-2604410

Medical Research Administration – tel.: (031) 260 4495; fax: (031) 260 4410;
e-mail : ethicsmed@ukzn.za.

INFORMED CONSENT

Consent to participate in the study: Effect of nutritional supplements on lean muscle mass, general health and disease progression of HIV positive lactating mothers and the ensuing effects on their infants including the impact of feeding mode on disease progression of the infected infants

We hope we have explained the research we are doing clearly. If there are any doubts or queries you would like us to answer before making a decision, please let us know.
We would like to have your permission to enroll your child and yourself into our study.

I _____ hereby consent to my child and myself being enrolled in the above study.
I have been given all the information I need to make a proper informed decision.
I understand this is a research and if at any time my child needs treatment for HIV, he/she will be withdrawn from the study and treated appropriately at the routine HIV clinic.
I can contact the study clinician Gurpreet Kindra at 031-3603693/0829631921 at any time if I have questions about the research or if I feel that I should like to withdraw from the study.
I can contact the Medical Research Office at the Nelson R Mandela School of Medicine at 031-260 4604 if I have questions about my rights as a research subject.

My participation is voluntary and I shall not be penalized for refusing consent or withdrawing from the study at any stage.
If I agree to participate, I will be given a signed copy of this document and the participant information sheet, which is a written summary of the research.
The research study, including the above information, has been described to me orally. I understand what my involvement in the study means and I voluntarily agree to participate**.**

_____ _____
Signature of Participant Date

_____ _____
Signature of Witness Date
(Where applicable)

_____ _____
Signature of Translator Date
(Where applicable)

Consent for storage of samples

Samples collected from yourself/your child will be saved for future molecular, virological and immunological research laboratory tests pertaining to HIV infection, which are not available at the present time. This may not benefit your child but may benefit future children/ adults exposed or infected with the virus. You have the right to withdraw your permission to use the blood for future research at any time and for any reason.

- ☐ I give permission for samples collected from my child and myself to be saved for future research
- ☐ I do not give permission for samples collected from my child and myself to be saved for future research

Signature: _____

Date: _____

Witness: _____

Statement by client:
Ref: Copy of consent document

I do wish for a copy of the consent document and it has been given to me:
_____

I do not wish for a copy of the consent document: _____

Appendix 4

Nutrition Study

Name: _____

PMTCT barcode: _____

MTCT Plus No: _____

Study Number: _____

EDD: _____

Feeding choice: _____

Randomisation if applicable: _____

Consent: _____
Pre-enrollment form

Maternal Questionnaire (adapted from WHO) **Date: _____**

Socio-demographic and socio-economic data

1. Date of birth: _____

2. Years of schooling: _____

3. Marital status: Single/Married/Living with partner

4. Reproductive health history:

(i) Menarche: _____

(ii) Duration of cycle: _____

(iii) Parity: _____

(iv) Abortions/Miscarriages: _____

(v) Deaths of children under 5 years: _____

(vi) No of living children: _____

(vii) H/O of being treated for any PID: _____

(viii) Previous contraception use: Yes/No

(ix) If yes, type of contraception: (i) Oral (ii) Depot (iii) Nuristerate (iv) Other_____

(x) Plans for post delivery Contraception: Yes/No/TL

5. No of people living in the household: _____

6. No of deaths in the household during the last 12 months: (include cause if known)

0-1 years _____ relation_____ 1-5 years_____ relation_____

7. Chief source of income for household☹underline main source)Self/ Partner-husband/ Parents/ Grandparent/ / Child support grant/Pension/Own disability Grant/ Other_____

8. Current Family H/O TB: _____

9. Access to water: (i) Home (ii) Public tap (iii) River (iv) other

10. Access to Electricity☹i) Yes (ii) No

11. Sanitation: (i) Waterborne (ii) Pit (iii) other

12. Food security (i) Yes (ii) No

13. Disclosure of HIV status to partner: (i) Yes (ii) No

14. If no, why not: _____

15. Whether partner's HIV status known: Unknown/Positive/Negative

Maternal health status (collected at ANC)

16. CD4 count during pregnancy; _____

17. WHO Clinical staging of HIV: _____

18. Symptoms or definitive diagnosis of tuberculosis: Yes/ No

19. WR result: non-reactive/ reactive, treated/ reactive- untreated

20. Complications during pregnancy: _____

21. Nutritional status:

5 Weight: _____

(ii)Height: _____

(iii)BMI: _____

(iv) Haemoglobin: _____

22. AFASS Criteria fulfilment based on above: Yes/No

Delivery form

Obstetric information

1. Date of delivery: _____

2. Time of delivery: _____

3. Duration of labour: _____

4. Type of delivery: NVD/Elective LSCS/ Emergency LSCS

5. Indication, if LSCS: _____

6. Premature rupture of membranes: Yes/No; if Yes, duration_____

7. Episiotomy or tear requiring stitches: Yes/No

8. ARV given to mother before delivery: Yes/No

9. If yes, specify: (i) sdNVP (ii) sdNVP+Combivir (iii) Combivir (iv) None

10. ARV given to child after delivery: Yes/No

11. If yes, specify: (i) sdNVP (ii) sdNVP+Retrovir (iii) Retrovir (iv) None

12. Gestational age: _____

13. Complications during delivery: _____

Birth history

1. Sex: _____

2. Birth weight: _____

3. HC: _____

4. Length: _____

5. Neonatal complications: Yes/No, if yes specify_____

6. Choice of feeds (0-6months): (i) Breast (ii) Formula (iii) Mixed

Maternal Enrollment form **Date: _____**

1.	Feeding choice	(i) Excl Breastfeeding (ii) Excl Formula (iii)Mostly Breast (iv) Mixed
2.	Randomization if breastfeeding	A B
3.	Oral thrush	
4.	Lymphnodes	
5.	Hepatomegaly	
6.	Splenomegaly	
7.	H/O fever	
8.	H/O diarrhoea	
9.	H/O Vomiting	
10.	H/O Vaginal discharge	
11.	Signs of PID	
12.	Skin lesions	
13.	Signs of LRTI	
14.	URTI	
15.	Neurological signs	
16.	H/O Admissions	
17.	Duration of hospital stay	
18.	Contraception	(i) Oral (ii) Depo Provera(iii) Nur-Isterate(iv) Other_____
19.	Condom usage	
20.	Other	
21.	Karoffsky score	
22.	WHO Staging	
23.	Morbidity score	
24.	Assessment	
25.	Management	
26.	Investigations	
27.	Medication	

Anthropometric assessment- Mother

Height: _____

Date	Wt 1	Wt 2	Mean	MUAC 1	MUAC 2	Mean	Triceps SF 1	Triceps SF2	Mean	BMI

Anthropometric assessment- Infant

Date	W1	W2	Mean	HC1	HC2	Mean	L1	L2	Mean	MUAC 1	MUAC 2	Mean	TSF 1	TSF2	Mean	BMI

Bioelectrical Impedance Analysis

Date					
Weight (kg)					
Age (years)					
Time					
Activity Level					
TBW%					
Normal TBW%					
TBW (lt)					
Normal TBW (lt)					
BMI					
Impedance: 5 kHz					
Impedance: 50 kHz					
Impedance: 100 kHz					
Impedance: 200 kHz					

Isotope Data

Date				
Weight				
Ambient temperature				
Time of last meal				
1st sample				
Time of ingestion of D20				
Did client eat/drink anything during waiting period, if yes, specify				
2nd sample				
Comments				

Infant feeding Questionnaire **Date:_____**

1. Compliance with choice: Yes/No

2. Elaborate reasons for change in feeding mode: _____

3. Family/Partner response to feeding mode: _____

4. If breast fed, please answer following:

(i) How soon after delivery was your infant first put to the breast? (in hours) _____

(ii) Did your infant receive anything to eat/drink before he was first put to the breast?: Yes/No/don't know

Food items given before infant was put to the breast for the first time and during the first 3 days of life

	Before any breast milk		Day 1		Day 2 to Day 3	
	Given	Reason	Given	Reason	Given	Reason
Breast milk						
Unsure if other food given						
Water or Glucose water						
Tea or Juice						
Formula						
Other milk						
Cereals or porridge (home prepared or commercial)						
Vegetables or fruits						
Other foods unspecified						
Other foods specified						
Pharmaceutical medicines						
Traditional medicines						

Self Reporting Questionnaire **Date: _____**

Question:	**Circle Answer**	
1. Do you often have headaches?	Yes	No
2. Is your appetite poor?	Yes	No
3. Do you sleep badly?	Yes	No
4. Are you easily frightened?	Yes	No
5. Do your hands shake?	Yes	No
6. Do you feel nervous, tense or worried?	Yes	No
7. Is your digestion poor?	Yes	No
8. Do you have trouble thinking clearly?	Yes	No
9. Do you feel unhappy?	Yes	No
10. Do you cry more than usual?	Yes	No
11. Do you find it difficult to enjoy your daily activities?	Yes	No
12. Do you find it difficult to make decisions?	Yes	No
13. Is your daily work suffering?	Yes	No
14. Are you unable to play a useful part in life?	Yes	No
15. Have you lost interest in things?	Yes	No
16. Do you feel like a worthless person?	Yes	No
17. Has the thought of ending your life been in your mind?	Yes	No
18. Do you feel tired all the time?	Yes	No
19. Do you have uncomfortable feelings in your stomach?	Yes	No
20. Are you easily tired?	Yes	No

SCORE 1 POINT FOR EVERY "YES" ANSWER

Refer any patient who scores 8 or more for a PSE interview

This score was determined by a pilot study of "normals" versus known psychiatric depressed/anxious patients

21. So you feel that somebody has been trying to harm you in some way?	Yes	No
22. Are you a much more important person than most people think?	Yes	No
23. Is there a problem or something unusual with your thinking?	Yes	No
24. Do you hear voices without knowing where they come from, or which other people cannot hear?	Yes	No
25. Have you ever had any fits?	Yes	No

If any of the questions 22-24 are marked "Yes", refer for PSE.

Use clinical judgement with question 21.

In addition, if the patient's behaviour seems definitely strange or unusual (e.g. hallucinating, unhappy or playful), refer for PSE.

Maternal follow up

Date						
1	Feeding choice	(i) Excl Breast (ii) Excl Formula (iii)Mostly Breast (iv) Mixed	(i) Excl Breast (ii) Excl Formula (iii)Mostly Breast (iv) Mixed	(i) Excl Breast (ii) Excl Formula (iii)Mostly Breast (iv) Mixed	(i) Excl Breast (ii) Excl Formula (iii)Mostly Breast (iv) Mixed	(i) Excl Breast (ii) Excl Formula (iii)Mostly Breast (iv) Mixed
2	Randomization if breastfeeding	A B	A B	A B	A B	A B
3	Oral thrush					
4	Lymphnodes					
5	Hepatomegaly					
6	Splenomegaly					
7	H/O fever					
8	H/O diarrhoea					
9	H/O Vomiting					
10	H/O Vaginal discharge					
11	Signs of PID					
12	Skin lesions					
13	Signs of LRTI					
14	URTI					
15	Neurological signs					
16	H/O Admissions					
17	Duration of hospital stay					
18	Contraception	(i) Oral (ii) Depo Provera (iii) Nur-Istrate(iv) Other_____	(i) Oral (ii) Depo Provera (iii) Nur-Isterate(iv) Other_____	(i) Oral (ii) Depo Provera (iii) Nur-Isterate(iv) Other_____	(i) Oral (ii) Depo Provera (iii) Nur-Isterate(iv) Other_____	(i) Oral (ii) DepoProvera (iii) Nur-Isterate(iv) Other_____
19	Condom usage					
20	Duration of menstrual cycle					
21	Other					
22	Karoffsky score					
23	WHO Staging					
24	Morbidity score					
25	Assessment					
26	Management					
27	Investigations					
28	Medication					

Paediatric Enrollment visit **Date: _____**

1.	Age	
2	Oral thrush	
3	Lymphnodes	
4	Hepatomegaly	
5	Splenomegaly	
6	H/O fever	
7	H/O diarrhoea	
8	H/O Vomiting	
9	H/O Ear discharge	
10	Skin lesions	
11	Signs of LRTI	
12	URTI	
13	Neurological signs	
14	H/O Admissions	
15	Duration of hospital stay	
16	Immunisation	
17	Other	
18	Assessment	
19	Developmental assessment	
20	WHO stage	
21	Management	
22	Investigations	
23	Medication	

Date: _____

Feeding Questionnaire for follow up visits

1. Change in feeding mode since last visit (0-6months): (i) Breast (ii) Formula (iii) Mixed

2. Elaborate reasons for change in feeding mode: _____

3. Date of change/ stopping breast feeding: _____

4. If breast fed, please answer following:

Food items given since last visit

			Duration
	Given	Reason	
Breast milk			
Unsure if other food given			
Water or Glucose water			
Tea or Juice			
Formula			
Other milk			
Cereals or porridge(home prepared or commercial)			
Vegetables or fruits			
Other foods unspecified			
Other foods specified			
Pharmaceutical medicines			
Traditional medicines			

Paediatric Follow up

1.	Date &age					
2	Oral thrush					
3	Lymphnodes					
4	Hepatomegaly					
5	Splenomegaly					
6	H/O fever					
7	H/O diarrhoea					
8	H/O Vomiting					
9	H/O Ear discharge					
10	Skin lesions					
11	Signs of LRTI					
12	URTI					
13	Neurological signs					
14	H/O Admissions					
15	Duration of hospital stay					
16	Immunisation					
17	Other					
18	Assessment					
19	Developmental assessment					
20	WHO stage					
21	Management					
22	Investigations					
23	Medication					

Truncated Denver Developmental Test

Evaluate for participant's age: Yes = 1 / No = 2 / Unable to assess = 3

Age 3 – 4 months:

Grasps rattle		
Puts hands together		Date:
Bears weight on legs		Signature:

Age 5 – 7 months:

Passes a cube from hand to hand		
Puts hands together		Date:
Bears weight on legs		Signature:

Age 8 – 10 months:

Thumb finger grasp		
Bangs two cubes together		Date:
Stands holding on		Signature:

Age 11 – 13 months:

Puts block in cup		
Bangs two cubes held in hands		Date:
Stands for 2 seconds		Signature:

Table 1.

REVISED WHO CLINICAL STAGING OF HIV/AIDS FOR ADULTS AND ADOLESCENTS

Primary HIV infection
Asymptomatic
Acute retroviral syndrome

Clinical stage 1
Asymptomatic
Persistent generalized lymphadenopathy (PGL)

Clinical stage 2
Moderate unexplained weight loss (<10% of presumed or measured body weight)
Recurrent respiratory tract infections (RTIs, sinusitis, bronchitis, otitis media, pharyngitis)
Herpes zoster
Angular cheilitis
Recurrent oral ulcerations
Papular pruritic eruptions
Seborrhoeic dermatitis
Fungal nail infections of fingers

Clinical stage 3
Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations
Severe weight loss (>10% of presumed or measured body weight)
Unexplained chronic diarrhoea for longer than one month
Unexplained persistent fever (intermittent or constant for longer than one month)
Oral candidiasis
Oral hairy leukoplakia
Pulmonary tuberculosis (TB) diagnosed in last two years
Severe presumed bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia)
Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis
Conditions where confirmatory diagnostic testing is necessary
Unexplained anaemia (< 8 g/dL), and or neutropenia (<500/mm3) and or thrombocytopenia (<50 000/ mm3) for more than one month

Clinical stage 4
Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations
HIV wasting syndrome
Pneumocystis pneumonia
Recurrent severe or radiological bacterial pneumonia
Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration)
Oesophageal candidiasis
Extrapulmonary TB
Kaposi's sarcoma
Central nervous system (CNS) toxoplasmosis
HIV encephalopathy

Conditions where confirmatory diagnostic testing is necessary:
Extrapulmonary cryptococcosis including meningitis
Disseminated non-tuberculous mycobacteria infection
Progressive multifocal leukoencephalopathy (PML)
Candida of trachea, bronchi or lungs
Cryptosporidiosis
Isosporiasis
Visceral herpes simplex infection

Cytomegalovirus (CMV) infection (retinitis or of an organ other than liver, spleen or lymph nodes)
Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis, penicilliosis)
Recurrent non-typhoidal salmonella septicaemia
Lymphoma (cerebral or B cell non-Hodgkin)
Invasive cervical carcinoma
Visceral leishmaniasis

Table 2

REVISED WHO CLINICAL STAGING OF HIV/AIDS FOR INFANTS AND CHILDREN

Clinical Stage 1
Asymptomatic
PGL

Clinical Stage 2
Hepatosplenomegaly
Papular pruritic eruptions
Seborrhoeic dermatitis
Extensive human papilloma virus infection
Extensive molluscum contagiosum
Fungal nail infections
Recurrent oral ulcerations
Lineal gingival erythema (LGE)
Angular cheilitis
Parotid enlargement
Herpes zoster
Recurrent or chronic RTIs (otitis media, otorrhoea, sinusitis)

Clinical Stage 3
Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations
Moderate unexplained malnutrition not adequately responding to standard therapy
Unexplained persistent diarrhoea (14 days or more)
Unexplained persistent fever (intermittent or constant, for longer than one month)
Oral candidiasis (outside neonatal period)
Oral hairy leukoplakia
Acute necrotizing ulcerative gingivitis/periodontitis
Pulmonary TB
Severe recurrent presumed bacterial pneumonia
Conditions where confirmatory diagnostic testing is necessary
Chronic HIV-associated lung disease including brochiectasis
Lymphoid interstitial pneumonitis (LIP)
Unexplained anaemia (<8g/dL), and or neutropenia (<1000/mm3) and or thrombocytopenia (<50 000/ mm3) for more than one month

Clinical Stage 4
Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations
Unexplained severe wasting or severe malnutrition not adequately responding to standard therapy
Pneumocystis pneumonia
Recurrent severe presumed bacterial infections (e.g. empyema, pyomyositis, bone or joint infection, meningitis, but excluding pneumonia)
Chronic herpes simplex infection; (orolabial or cutaneous of more than one month's duration)
Extrapulmonary TB
Kaposi's sarcoma
Oesophageal candidiasis
CNS toxoplasmosis (outside the neonatal period)
HIV encephalopathy

Conditions where confirmatory diagnostic testing is necessary
CMV infection (CMV retinitis or infection of organs other than liver, spleen or lymph nodes; onset at age one month or more)
Extrapulmonary cryptococcosis including meningitis
Any disseminated endemic mycosis (e.g. extrapulmonary histoplasmosis, coccidiomycosis, penicilliosis)
Cryptosporidiosis
Isosporiasis
Disseminated non-tuberculous mycobacteria infection
Candida of trachea, bronchi or lungs
Visceral herpes simplex infection
Acquired HIV associated rectal fistula
Cerebral or B cell non-Hodgkin lymphoma
Progressive multifocal leukoencephalopathy (PML)
HIV-associated cardiomyopathy or HIV-associated nephropathy

Table 3

Karnofsky Performance status score

The Karnofsky score is another method which measures patient performance of activities of daily living. The score has proven useful not only to follow the course of the illness (usually progressive deficit and ultimately death), but also a prognosticator: patients with the highest (best) Karnofsky scores at the time of tumor diagnosis have the best survival and quality of life over the course of their illness.

SCORE FUNCTION

100 Normal, no evidence of disease
90 Able to perform normal activity with only minor symptoms
80 Normal activity with effort, some symptoms
70 Able to care for self but unable to do normal activities
60 Requires occasional assistance, cares for most needs
50 Requires considerable assistance
40 Disabled, requires special assistance
30 Severely disabled
20 Very sick, requires active supportive treatment
10 Moribund

Multivitamin supplement supplied to all mothers as part of national PMTCT guidelines

Pregi-Forte 30; Manufacturer: Portfolio Pharmaceuticals (Pty) Ltd, Johannesburg 2001

Composition

Vitamin A:	3000 I.U.
Vitamin B1:	3 mg
Vitamin B2:	2 mg
Pyridoxine:	1mg
Vitamin B12:	2 µg
Ascorbic acid:	50 mg
Vitamin D3:	400 I.U.
Calcium:	90 mg
Copper:	0.15 mg
Iron:	10 mg
Folic acid:	795 µg
Iodine:	0.01 mg
Magnesium:	3 mg
Manganese:	0.05 mg
Niacinamide:	10 mg
Potassium:	0.84 mg
Zinc:	5 mg

SOP: Preparation of D2O samples for Body composition

1. Aliquot 30g samples of D_2O into sterile glass bottles.
2. Record temperature of the room on the day of preparation of the samples
3. Keep the prepared bottles in an airconditioned room preferably as the temperature needs to be stable (to prevent evaporation of D_2O)

SOP: Saliva Sample collection for Body composition

1. Ask the subject to be fasting for at least 1 hour before collection of the baseline sample.
2. Wash hands thoroughly and dry them.
3. Give a wad of cottonwool/dental swabs to the subject to chew in her mouth till it's quite wet. Then ask her to roll it in her mouth into an oblong shape if possible.
4. Wear a pair of gloves and take a 10 ml syringe and open it, ask the subject to place the cotton wool in the barrel of the syringe.
5. Then replace the plunger and try and squeeze the cotton wool as much as possible into a cryotube, you need to obtain about 3-4ml of saliva.
6. Place the cryotube into a -20 – 80 ^{0} C freezer, if you are in the field, you can use an ice box or cooler box till you can get to a freezer, its important for the samples to remain in a cold environment so that there is no evaporation of the saliva.
7. Now give the dose of D_2O to the subject, record the time.
8. Wash your hands after handling the D_2O as you can contaminate other samples.
9. Ask the subject to remain fasting for 4 hours.
10. Take the next saliva sample in the same way 3 hours after giving the D_2O, record the time again.
11. Repeat saliva sample collection at 4 hours again and record the time.

Equipment needed

1. Deuterium
2. Sterile glass Bottles around 50 ml bottles
3. Weighing scale to weigh out the Deuterium
4. Room thermometer
5. Dental swabs
6. 4ml cryotubes
7. 10ml syringes
8. Cooler box

Appendix 7

RAF/6/039 STANDARD OPERATING PROCEDURE FOR ANALYSIS OF DEUTERIUM ENRICHMENT BY FTIR

1.0 Purpose

1.1 This SOP describes the procedure for analysis of deuterium enrichment by Fourier transform infrared spectrometry (FTIR) in saliva samples collected from studies of body composition and human milk intake assessed using deuterium dilution techniques.

2.0 Scope

2.1 This procedure should be followed by all participants in RAF6039 and other projects funded by the International Atomic Energy Agency (IAEA).

2.2 Any queries comments or suggestions relating to this SOP should be address to a technical officer at the Nutritional and Health Related Environmental Studies section of the Division of Human Health, IAEA, Vienna, Austria (C.Slater@iaea.org).

3.0 Safety requirements

3.1 Laboratory coats and gloves must be worn at all times in the work area.

3.2 Wear closed, flat shoes.

3.3 Long hair should be tied back.

3.4 The work area should be clean and tidy.

3.5 No eating, drinking or applying of cosmetics in the laboratory.

4.0 Associated documents

4.1 Local laboratory safety rules.

4.2 Local rules for receipt and traceability of samples.

4.3 FTIR manufacturer's operating manual.

4.4 Study report forms and SOP's.

4.5 Standard Operating Procedure for preparation of deuterium oxide doses.

4.6 Standard Operating Procedure for saliva sampling

5.0 Notes

5.1 **The FTIR Laboratory**:

The FTIR should be sited in a well-ventilated room to avoid build-up of CO_2 in the atmosphere. Ideally the room should be air-conditioned with controlled temperature and humidity. The bench on which the FTIR is placed should not be subject to vibration from nearby equipment or external sources.

Do not leave the cover of the sample chamber open for longer than the time it takes to put the cell in the holder.

Do not move the FTIR once it has been installed.

5.2 Cleaning the FTIR

Use a water-dampened cloth to wipe the exterior of the instrument to keep the FTIR dust free. It is not advisable to wipe the inside of the sample compartment.
If spillage from the cell occurs inside the compartment, clean up immediately with an absorbent lint-free cloth.

5.3 Care of the FTIR cell

Do not dismantle the cell unless there is a problem. The spacers are very fragile. If they are damaged the path length will change and the seal will be broken.
Fill the cell with clean water before storing. Replace water every week if it has not been used. Alternatively, air dry the cell using a syringe.

5.4 **Dose of D_2O when samples are analysed by FTIR**

The 162tabilize162ed dose for adults is 30 g 99 atom % deuterium oxide (D_2O), which gives a target enrichment in body water of 900 (range 500-1500) mg D_2O/kg body water after the dose has equilibrated with body water (Day 1 in human milk intake methodology).

5.5 **Limit of detection**

The limit of detection is 100 mg/kg mg D_2O /kg body water.

6.0 Quality Control

6.1 All measurements are made with reference to gravimetrically prepared standards.

6.2 Label bottles carefully with date and calculated D_2O enrichment.

6.3 The balance used for preparing standards must be on a stable table away from open windows and draughts. Switch off air conditioning units when using the analytical balance.

6.4 Balances must be level and calibrated before use.

6.5 All glassware must be clean and dry before use.

6.6 Keep a laboratory note book to record details of weights when making standards, and keep a record of all samples analysed, including QC checks.

6.7 Analyse standards as samples at the beginning, middle and end of each day.

7.0 Equipment

7.1 Shimadzu FTIR running under IR Affinity software

7.2 Liquid cell with 100 μm path length and calcium fluoride window

7.3 Centrifuge with buckets to take sample vials, ideally refrigerated (1000 *g*)

7.4 Electronic balance weighing to 0.0001 g

7.5 Electronic balance weighing to 0.1 g

7.6 Voltage stabilizer/UPS for all electronic equipment (electronic balances/FTIR)

7.7 Racks for sample vials

8.0 Consumables

8.1 **Deuterium oxide (99.8 atom % ^2H)**

8.2 **Supply of local drinking water**

8.3 **250 mL borosilicate glass bottles with PTFE-lined screw caps for the aliquots of the calibration standard and local drinking water used as "working standards" on a daily basis**

8.4 **100 mL borosilicate glass bottles with PTFE-lined screw caps for storing standards (calibration curve)**

8.5 **Labels for bottles**

8.6 **Permanent ink pens for writing on labels**

8.7 **1 mL disposable plastic syringes with Luer tip for filling FTIR cell**

8.8 **Paper tissues/absorbent paper**

8.9 **Lens paper to clean window of FTIR cell**

8.10 **Volumetric flasks (1 L, 100 mL or 50 mL) for making calibration standards**

8.11 **Automatic pipettes plus tips (1mL, 200 µL, 20 µL) for making calibration standards**

8.12 **Wash bottle for filling volumetric flasks**

9.0 Procedures: Preparation of the calibration standard

A large volume of a calibrating or standard solution of approximately 1000 mg/kg (ppm), or 1 g/L should be prepared (gravimetrically) by weighing 99.8 atom % deuterium oxide and diluting in normal drinking water from the region. Do not use distilled water to make the calibration standard. Distilled water is subject to fractionation.

9.1 Using an analytical balance (accurate to 0.0001 g), weigh a clean, dry 50 mL volumetric flask with its stopper in place, or another similar container e.g. a clean, dry glass bottle with a cap.

9.2 Add a small volume (~20 mL) of drinking water to the flask, replace the cap and weigh again.

9.3 Add 1 g of 99.8 atom % deuterium oxide to the bottle. If you are using an adjustable pipette to transfer 1 g D_2O, then the volume selected should be 0.9 mL, as the density of deuterium oxide is higher than water (1.105 g/mL and 1.000 g/mL respectively at 25˚C). Replace the stopper or cap to avoid losses by evaporation, and note the weight. Calculate the weight of D_2O in the bottle.

9.4 Weigh a clean dry 1 L volumetric flask with its stopper. At this stage a balance weighing to 0.1g can be used.

9.5 Quantitatively transfer the water from the 50 mL container into the 1 L volumetric flask using a funnel. Add local drinking water to the smaller container and pour it into the larger container. Repeat this at least three times to ensure that all the deuterium oxide is transferred. Be careful not to spill any.

9.6 Add local drinking water to the 1 L volumetric flask up to the mark. Replace the stopper and weigh again.

9.7 After noting the weight, transfer the calibration standard to a clean, dry glass bottle with a PTFE lined screw cap.

9.8 Keep a similar volume of the local drinking water to use as a zero-standard or blank to measure the background spectrum.

9.9 Calculate the enrichment of the calibration standard as follows:

9.9.1 If (A) is the weight of 99.8 atom % deuterium oxide (D_2O), (B) is the weight of drinking water plus D_2O in the 1L flask, then the weight of added drinking water is (B-A).

9.9.2 Enrichment of D_2O in the calibration standard = $A/(B-A) \times 10^6$ mg/kg

See Appendix 1 for example calculation.

9.10 Transfer the contents of the volumetric flask into four 250 mL borosilicate glass bottles with PTFE-lined screw caps. Save a similar quantity of the water used to make the dilution. Use one of each of these as "working standards". The remainder will remain unopened until required. This has the advantage of only exposing a small portion of the calibration standard to the atmosphere at any time.

9.11 Write the date, D_2O enrichment and the initials of the person who prepared the standard on the label.

9.12 Shelf-life of the calibration standards

The shelf-life of the calibration standards will depend on the quality of the local drinking water. The bottles should be stored in a cool dark place out of direct sunlight, but not in the same refrigerator as the highly enriched (99.8 atom %) deuterium oxide. Wrapping bottles in aluminium foil helps to protect the contents from light.

10.0 Accuracy and precision: preparation of a standard curve

10.1 The accuracy and precision of deuterium analysis over the range of enrichments likely to be encountered should be checked periodically (as a training exercise and whenever the FTIR has not been used for 6 months or more) using gravimetrically prepared standards. The enrichment should range from 0 (natural abundance drinking water) to 2000 mg/kg, i.e. an enrichment above that likely to be encountered in saliva samples.

10.2 Standards should be made (in 100 mL local drinking water) according to the following table. The deuterium oxide can be pipetted into the volumetric flask (column 2), but it must be accurately weighed (column 3). Also note the weight of the drinking water added to make up the volume (column 4). The actual enrichment (mg/kg) can be calculated from the weights as described previously.

PREPARATION OF FTIR STANDARDS

Target enrichment (mg/kg ^2H_2O)	µL 99 atom % ^2H_2O	Weight ^2H_2O (g) to 4 decimal places	Weight drinking water added (g)
0	**0**		
100	**10**		
200	**20**		
400	**40**		
600	**60**		
800	**80**		
1000	**90**		
1500	**140**		
2000	**180**		

10.3 **Standards should be analysed in triplicate according to the procedure described in sections 11 and 12.**

10.4 **Plot the calculated enrichment on the x-axis and the measured enrichment on the y-axis of the calibration curve (see Appendix 2).**

10.5 **Accuracy is determined from the gradient and intercept of the linear regression line through the data. The gradient should be close to 1 (>0.95) and the intercept should be close to 0 (-0.5 to + 20). If not, there is a problem with the weighing, with the calculations or with the analysis. Check the data input and, if necessary, start again and make new standards.**

10.6 Precision is the standard deviation (SD) of repeated measures of the same sample. Precision is usually expressed as the coefficient of variation (CV), which is the standard deviation expressed as percent of the mean, SD/Mean x 100. The CV should be less than 1%.

10.7 Within-day and between-days precision of analysis can be determined by repeated measures of the same standard.

11.0 Filling the FTIR cell

11.1 **Bottles containing standards should be inverted before opening to mix any condensation on the side of the bottle or in the cap into the main contents of the bottle. This is because the condensate is fractionated relative to the water.**

11.2 **Saliva samples must be completely thawed before analysis (ice is fractionated relative to liquid water). The vials containing specimens of saliva should be centrifuged for 10 minutes at 1000 *g* (with the caps on) to move any condensation in the lid down into the bulk of the specimen, and to remove bubbles.**

11.3 Clean the window of the cell with lens (lint-free) tissue before starting.

11.4 The capacity of the cell is approximately 150 μL. By pushing through 1 mL saliva or reference water, traces of the previous sample are removed.

11.5 Fill 1 mL disposable plastic syringe with sample (standard or saliva).

11.6 Firmly press folded absorbent paper over the exit port to absorb excess sample and prevent ingress of air or use a second syringe to remove excess.

11.7 Fill the cell by firmly pushing the syringe plunger.

11.8 Remove excess/splashes from the outside of the cell window using absorbent paper.

11.9 Check for bubbles by holding the cell up to a light.

11.10 If there are visible bubbles in the cell, add more sample as described above until all of the bubbles have been pushed out.

11.11 Measure the absorbance from 2300-2900 cm^{-1} as described in section 12.

11.12 Remove the sample using the same syringe that was used for filling. Discard the syringe.

11.13 Use a new syringe for each sample to avoid cross-contamination.

11.14 Next Sample: Repeat from step 1.

11.15 Analyse the 0 and 1000 mg/kg standards as samples in the middle and at the end of the batch to check the calibration of the FTIR.

11.16 When all the samples have been analysed, rinse the cell with drinking quality water and fill with water or dry by pushing air through with a syringe before storing.

Procedure for filling the cell. Fill the cell using a 1 mL syringe, check for bubbles by holding the cell up to a light, place the cell in the sample chamber of the FTIR.

12.0 Good Laboratory Practice

12.1 Care must be taken to avoid fractionation during sample analysis. Evaporation causes fractionation. The vapour contains less deuterium than the liquid water. Evaporative losses result in an increase in the deuterium content of the water left behind. See Appendix of IAEA handbook on Stable Isotope Technique to Assess Human Milk Intake in Breastfed Babies for more information on fractionation.

12.2 Do not pour standards into beakers. Evaporation causes fractionation. Keep in a screw cap bottle and sample from the bottle.

12.3 Do not leave bottles and vials open to the atmosphere (fractionation). Remove the cap for only the time required to fill the pipette or syringe.

12.4 Do not put excess back in the bottle. Discard in the waste.

12.5 Precautions must be taken to avoid cross-contamination between samples. Do not use syringes for more than one sample when filling cells.

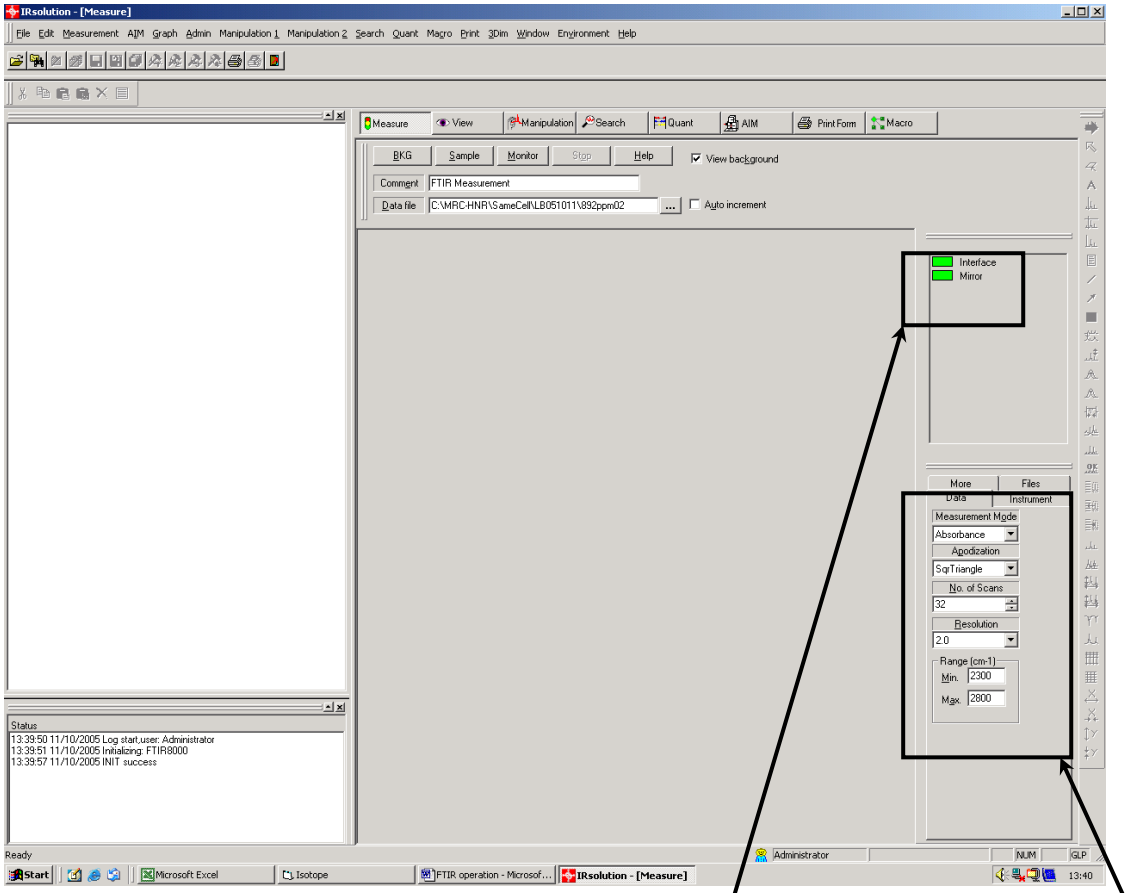
12.6 Do not touch the computer keyboard while wearing gloves. If possible work in pairs. One person can fill the cell and put it into the chamber of the FTIR. The second person can work at the computer and record details in the laboratory notebook.

12.7 Dispose of excess saliva in disinfectant and incinerate syringes with clinical waste.

13.0Preparing the FTIR for measurement

13.1 The FTIR should be switched on 30 minutes before using to allow the electronics to 167tabilize.

13.2 When the Shimadzu IRSOLUTION software is started this screen will be seen.

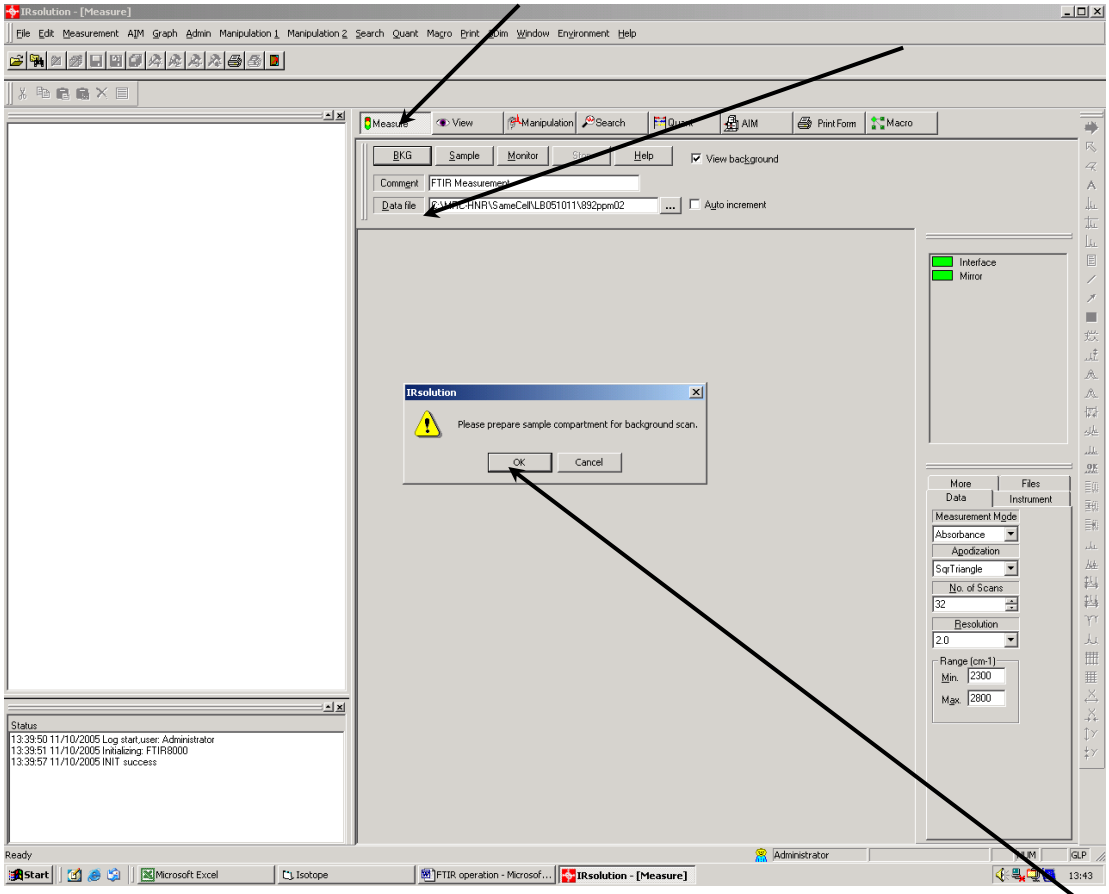


13.2.1 Firstly ensure that the FTIR is operating correctly. There should be two green segments indicating that both the interface and the mirror are working properly.

13.2.2 The spectrometer settings should be checked. Ensure that the following are set:

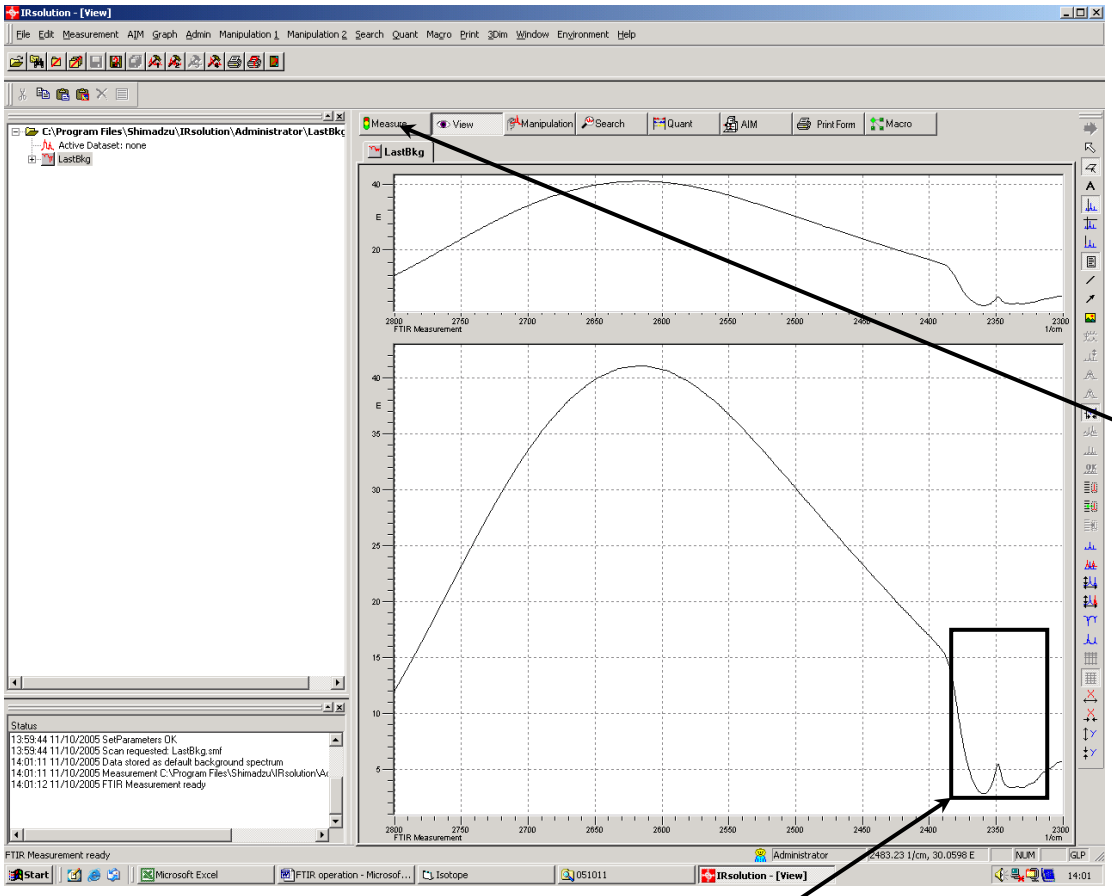
Measurement mode:	Absorbance
Apodization:	SqrTriangle
No of scans:	32
Resolution:	2.0
Range (cm^{-1}):	
Minimum	2300
Maximum	2900

13.2.3 The ‘Background’ unenriched (natural abundance) water spectrum can now be taken. Click on ‘Measure’, and then ‘Background’



Fill a cell with unenriched water, place in the FTIR sample holder, and click on ‘OK’.

It will take the FTIR about 90 seconds for the measurement, during which time the currently averaged spectrum will be displayed. When acquisition is complete a background with this characteristic shape should be displayed.



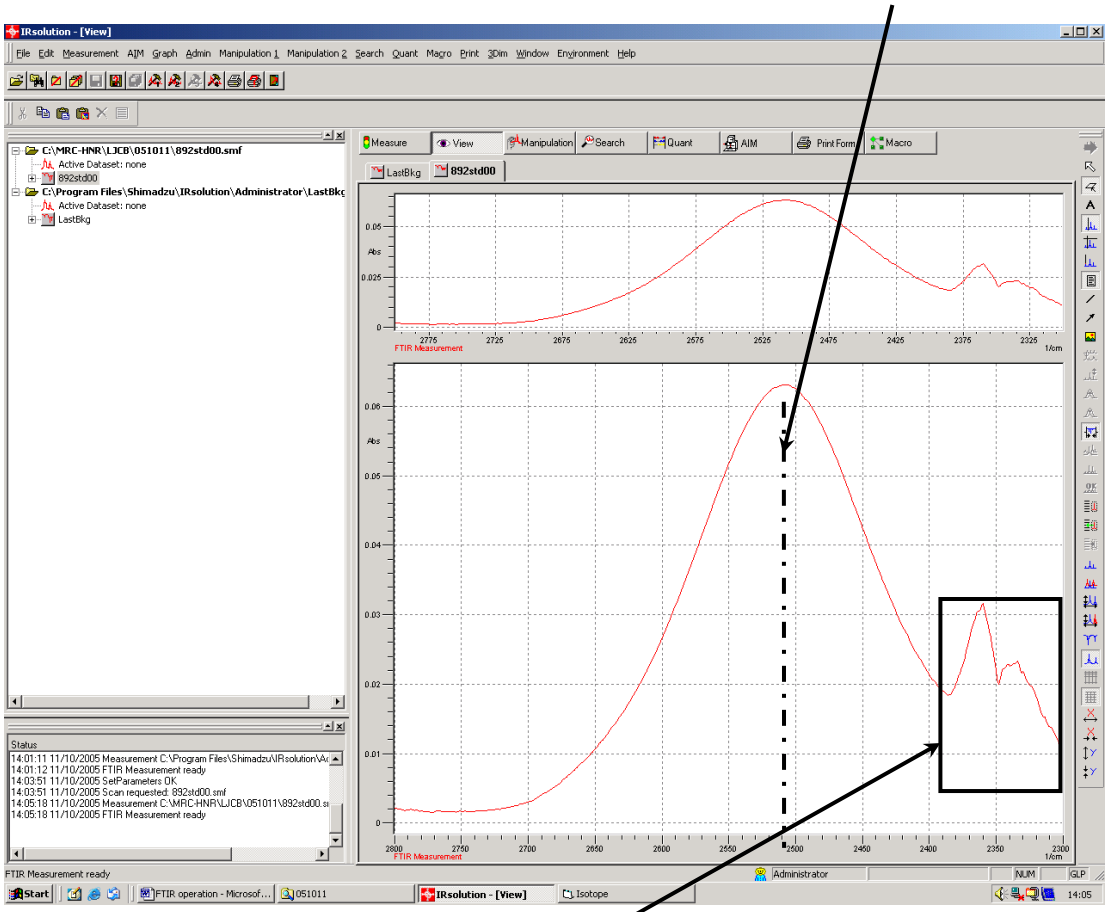
Note the doublet due to atmospheric carbon dioxide.

If the peak is not smooth, the desiccant needs to be changed.

13.2.4 Next prepare to calibrate the instrument. Use the calibration standard with deuterium enrichment about 1000 mg/kg (ppm) deuterium oxide. Obtain the spectrum using the 'Measure' button, followed by 'Sample'. Remember to set a suitable filename.

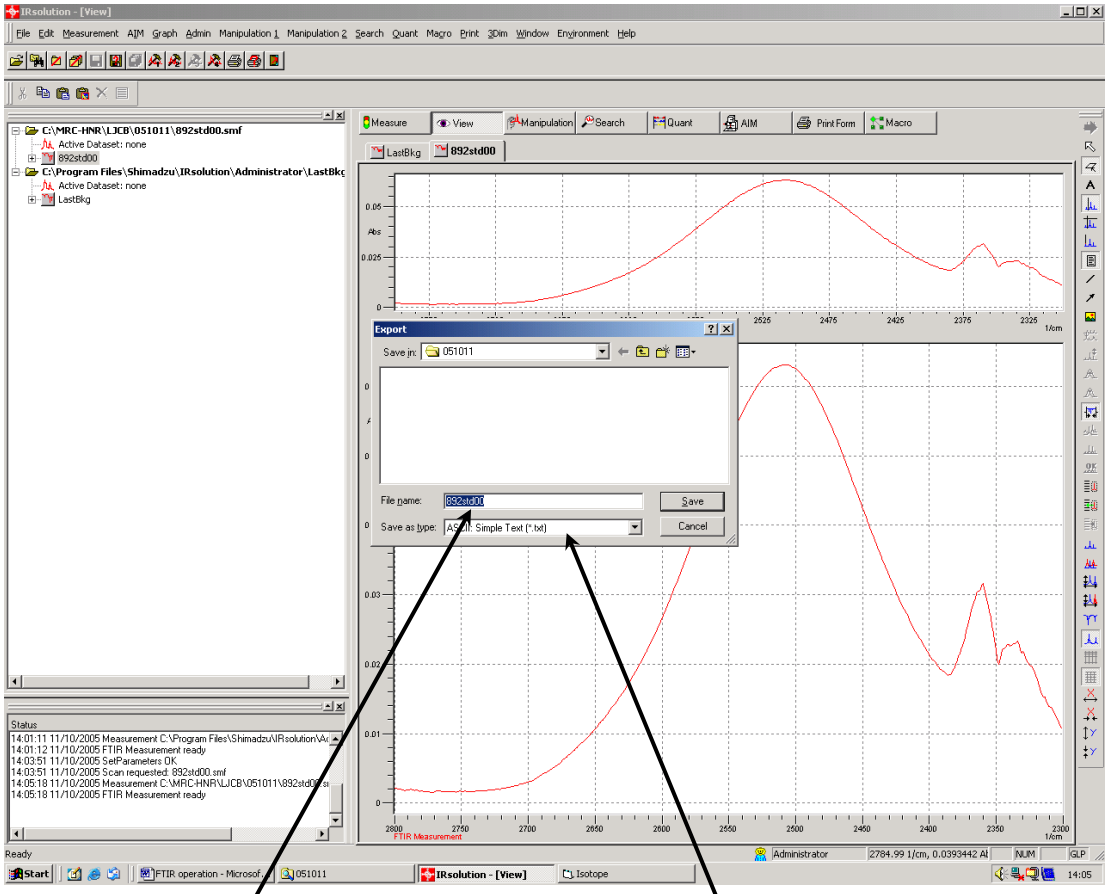
After about 90 seconds the measurement will be complete.

The peak due to deuterium should have a maximum at about 2510cm^{-1}.



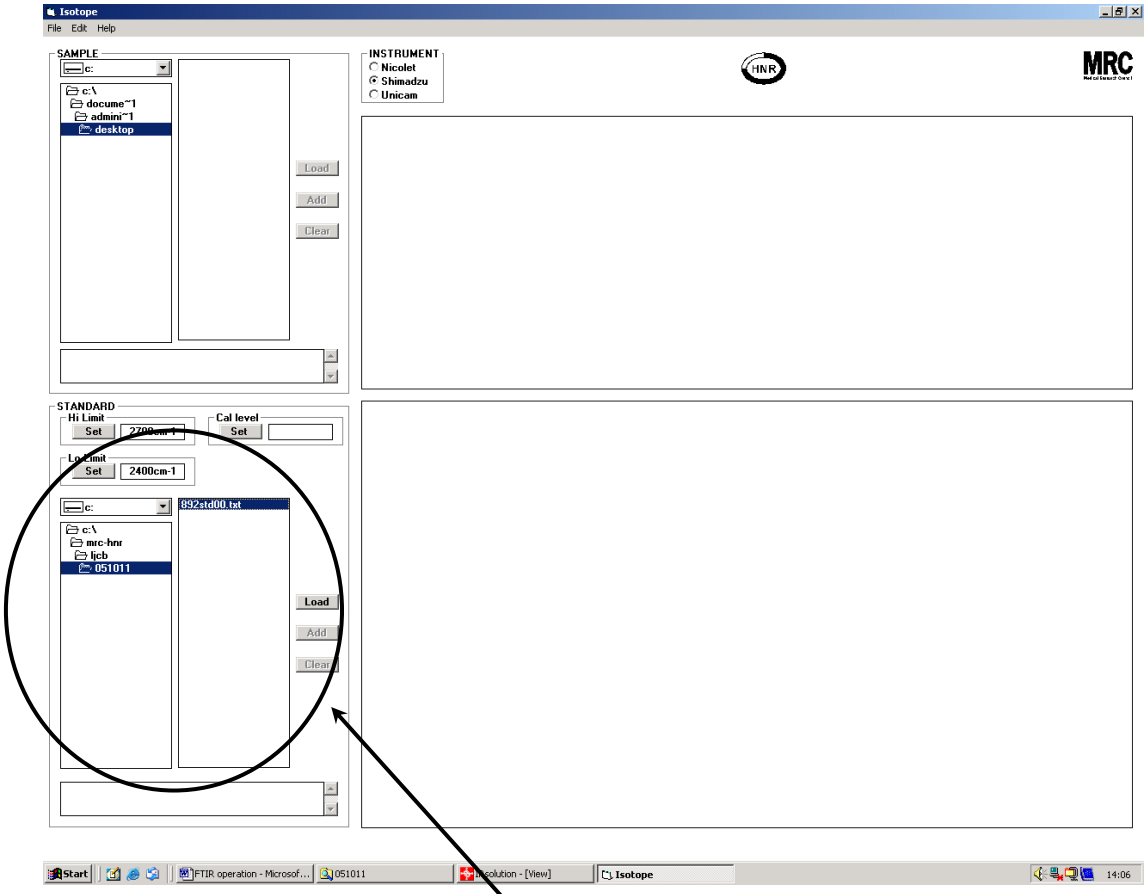
There will be interference from CO_2 on the low energy (right hand) side of the peak. This might be either positive or negative (in the example shown it is positive).

13.2.5 This spectrum should now be saved to disc in a form that can be read by the special MRC software. Choose ‘File’ from the main menu bar at the top, and then ‘Export’.



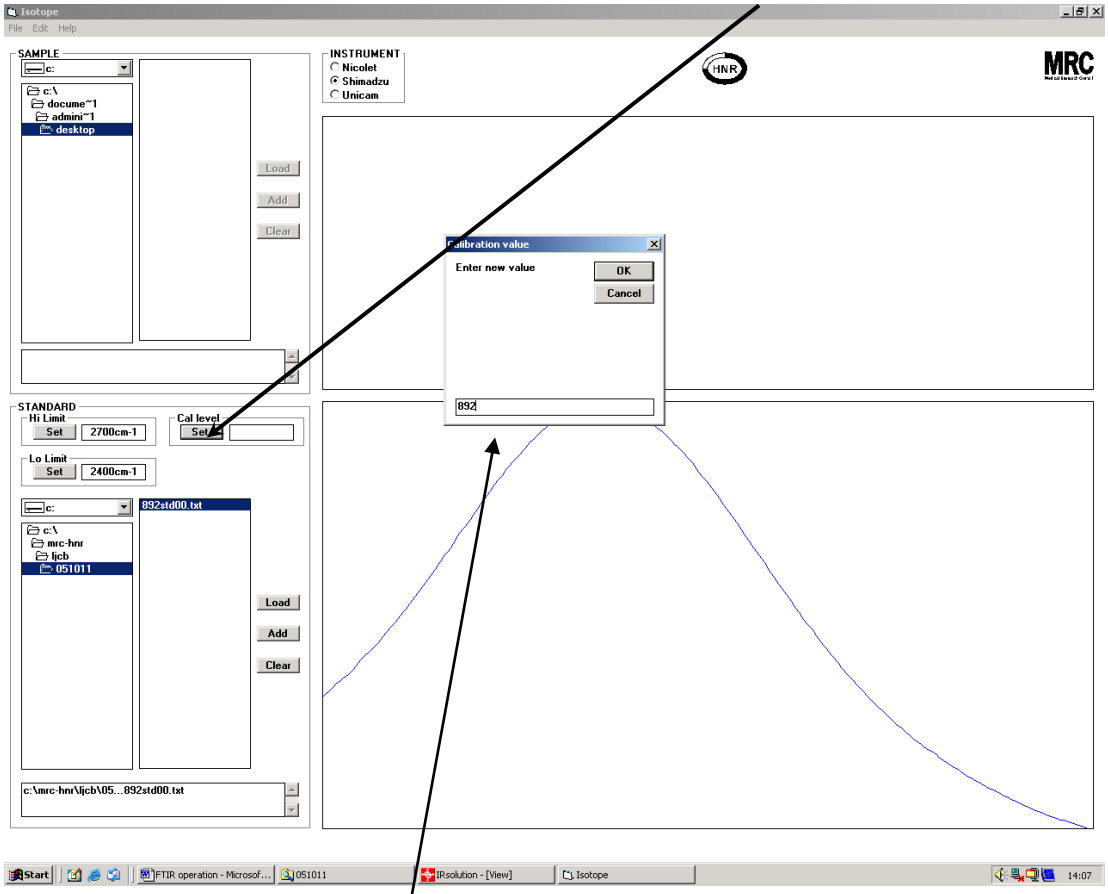
Choose a filename. Ensure that the exported filetype is ‘ASCII Simple Text (*.txt)’. Then click on ‘Save’.

13.2.6 Now launch the “isotope.exe” programme.



In the section for the standard select the file that has just been exported, and Load it.

13.2.7 Now set the calibration level. Click on the “Cal level set” button

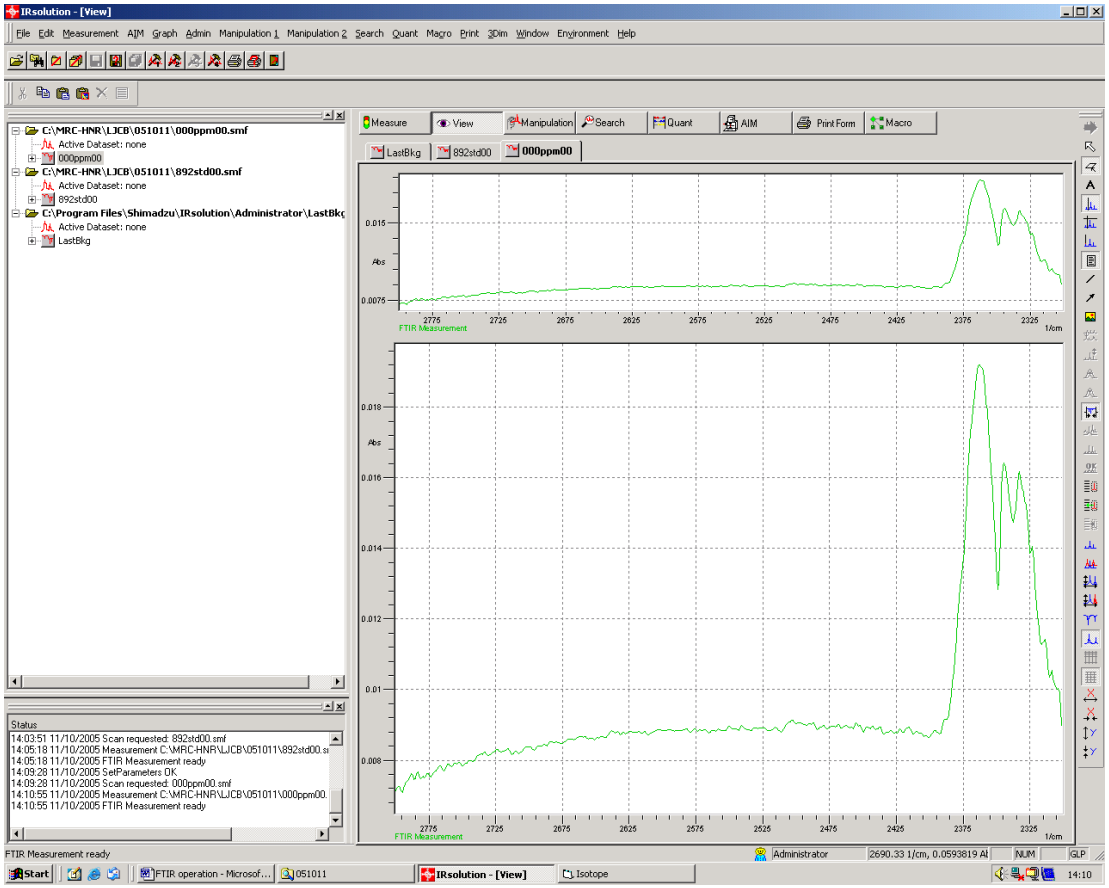


Enter the calculated enrichment of the calibration standard in the dialogue box and then click on “OK”.

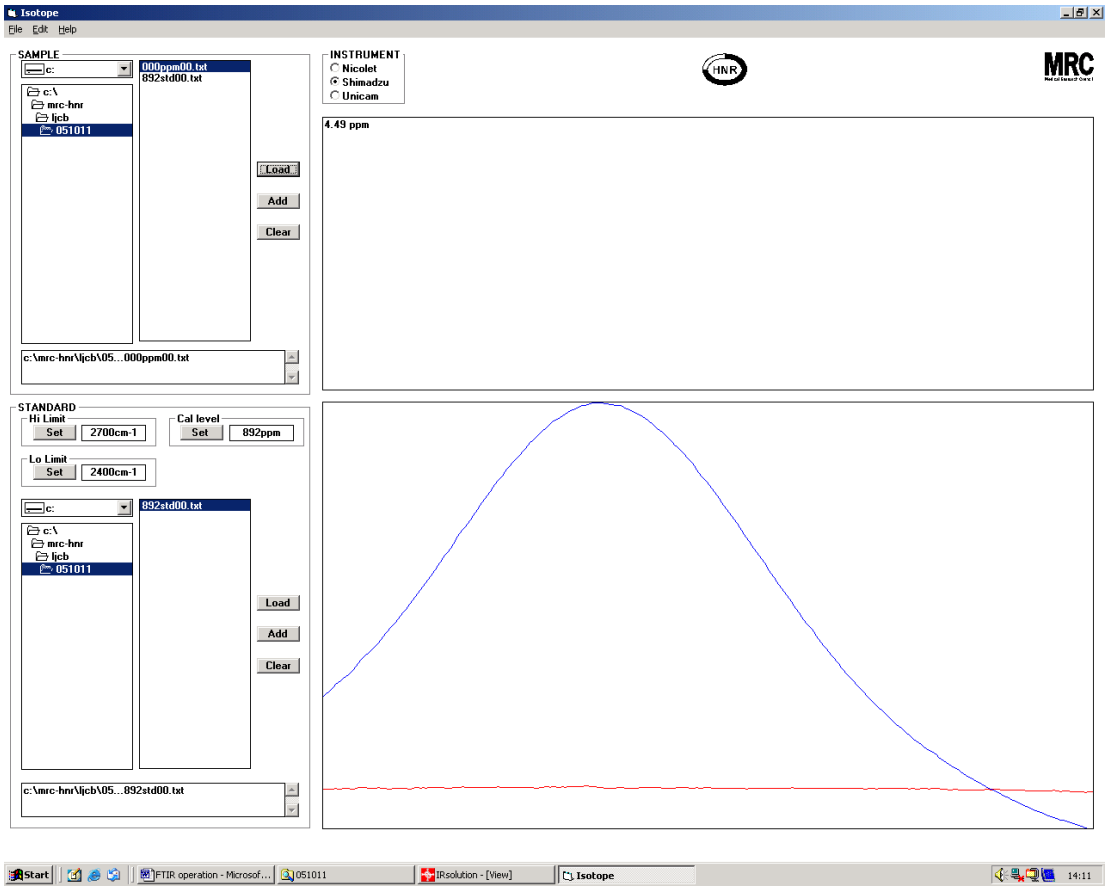
The system is now ready to make measurements of deuterium isotope composition of water or saliva samples.

13.2.8 To check that the reference and calibrant levels have been correctly set the following two steps should be performed.

Refill the sample cell with the background unenriched water sample, and measure it as though it were a sample. The spectrum should be almost featureless, apart from the possibility of CO_2 interference.



Now export this file and read it into the “isotope.exe” software.



The reported concentration of D_2O should be small, in the range –10 to +10 mg/kg (ppm).

This is the most difficult measurement to make since it is heavily influenced by any slight imperfection in sample cell filling.

Now re-measure the calibration standard water in the same way. The answer obtained should be within 1% of the set value (i.e for 1000 mg/kg (ppm) should lie between 990 and 1010 mg/kg.

14.0 Supporting information:

14.1 Excel spreadsheet for calculating human milk intake

14.2 IAEA handbook on Stable Isotope Technique to Assess Human Milk Intake in Breastfed Babies

15.0 History review:

15.1 This draft was prepared at the IAEA Regional (AFRA) Training Course on Standard Operating Procedures (SOP) for Isotope Techniques in Infant and Young Child Nutritional Status, Dare es Salaam, United Republic of Tanzania, 17-21 August 2009.

Appendix 1

Example calculation of enrichment of standards (Section 9.9)

Calculate the enrichment of the calibration standard as follows:

- **If (A) is the weight of 99.8 atom % deuterium oxide (D_2O), (B) is the weight of drinking water plus D_2O in the 1L flask, then the weight of added drinking water is (B-A).**

- **For example, weight 99.8 atom % deuterium oxide (D_2O) = 1.0015 g (A)**

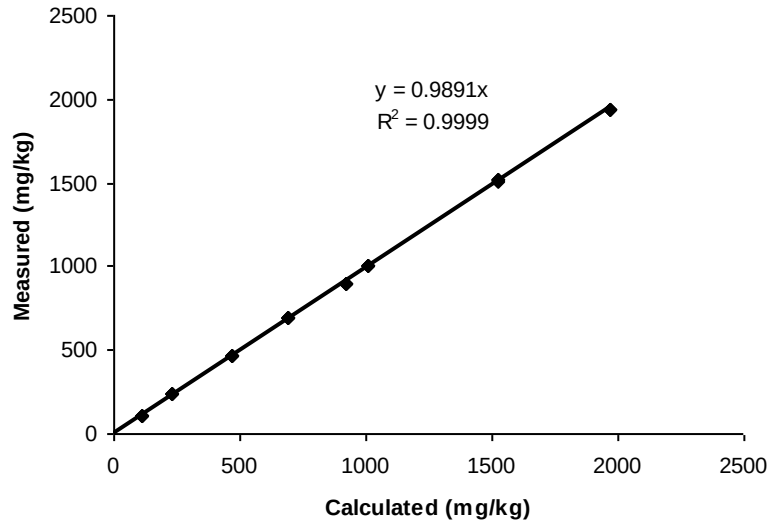
  Weight of drinking water plus D_2O in the 1L flask = 1000.1 g (B)
- **Then the weight of added drinking water = 1000.1 g -1.0015 g**

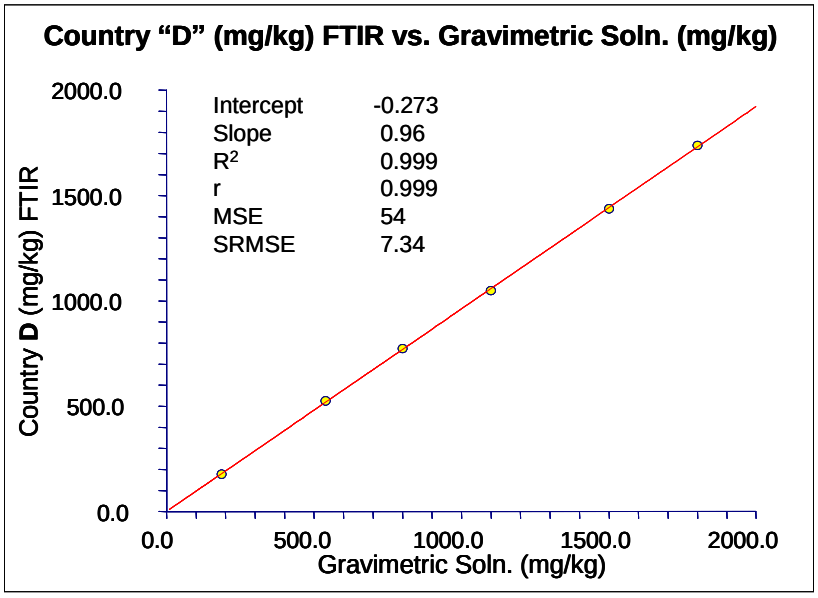
  = 999.0985 g (B-A)
- **Enrichment of D_2O in the calibration standard = A/(B-A) x 10^6 mg/kg**

  = 1.0015 g / 999.0985 g x 10^6 mg/kg
  = 1002 mg/kg (ppm)

Note: 1 mg/kg = 1 mg/L as the density of H_2O is 1.0 kg/L at 25°C, therefore the calibration standard is approximately 1000 mg/L.

Appendix 2
Example standard curves





Appendix 8

24-HOUR RECALL SUMMARY SHEET (SHEET 1)

DATE OF BIRTH: …….

DAY OF THE WEEK: ……DATE:…….

STEP 1 Foods/ drinks eaten/ drank during the day	STEP 2 Forgotten foods/ drinks

What you ate and drank yesterday, was it……? (Mark the appropriate block with an X)

The same as usual?	More than usual?	Less than usual?
Reason: …….		

What type of milk is usually used in your home? ……

What type of butter or margarine is usually used in your home? …….

What type of oil is usually used in your home? ……....

Are you taking any nutritional supplements? ……..…….

Are you taking any vitamins/ minerals? ……..……..

24-HOUR RECALL RECORDING SHEET FOR STEP 3 (SHEET 2)

Time	Any comment on time	Food Item	Detailed description of the item as well as preparation	Detailed description of portion size	Code	Weight (gram)

Appendix 9

Normal reference ranges for haematology, biochemistry and micronutrient levels

Haematology (source: NHLS)

Adult females

Haemoglobin:	11.5-13.5 g/dL
Haematocrit:	37-49 %
MCV:	79.1-98.9 fL
WCC:	3.92-9.88 $x10^9$/L
Lymphocyte count:	1.0-4.0 $x10^9$/L
Platelets:	178-400 $x10^9$/L

Infants (6 months)

Haemoglobin:	10.5- 13.7 g/dL
Haematocrit:	31-41%
MCV:	69-89 fL
WCC:	6-17.5 $x10^9$/L
Lymphocyte count:	4-10.5 $x10^9$/L
Platelets:	140-400 $x10^9$/L

Biochemistry (source: NHLS)

Total protein:	60-80 g/L
Albumin:	32-50 g/L

Normal serum micronutrient levels (source: NIRU, MRC, Cape Town)

Retinol:	>30 µg/dL (adults)
Zinc:	65 µg/dL
Folate:	>3 ng/mL
B12:	160-970pg/mL <120 pg/mL (deficiency)

Normal Breastmilk macronutrient and micronutrient levels

Age of infant:	**2/52**	**3/12**	**6/12**
Energy (kcal/g):	0.67	0.67	0.67
Protein (g/L):	11	9	8
Lactose (g/dL):	6.2	7.3	7.3
Fat (g/L):	3.6	3.8	3.8
Retinol (µmol/L):	1.7	1.7	1.7
Zinc (mg/L):	2.1	1.5	1

Appendix 10

GEE models for anthropometric and body composition measures comparing the breastfeeding control mothers to the formula feeding mothers

Table 6c: Parameter Estimates for GEE model for weight change from 2 weeks to 9 months post delivery

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	46.146	5.2655	35.826	56.467	76.805	1	.000
[Group=2] Breastfeeding control	.100	1.9154	-3.655	3.854	.003	1	.959
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	-.039	.0252	-.089	.010	2.422	1	.120
[Group=2] * Timeinweeks	.023	.0357	-.047	.093	.422	1	.516
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.785	.1893	.414	1.156	17.199	1	.000
CD4	.002	.0015	-.001	.004	1.086	1	.297
(Scale)	142.178						

Dependent Variable: Weight
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4
a Set to zero because this parameter is redundant.

Table 6d: Parameter Estimates for GEE model for BMI change from 2 weeks to 9 months post delivery

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	19.390	2.1294	15.217	23.564	82.918	1	.000
[Group=2] Breastfeeding control	.002	.7880	-1.542	1.546	.000	1	.998
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	-.015	.0102	-.035	.005	2.152	1	.142
[Group=2] * Timeinweeks	.010	.0144	-.018	.038	.492	1	.483
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.295	.0764	.145	.445	14.921	1	.000
CD4	.001	.0005	.000	.002	1.277	1	.258
(Scale)	23.734						

Dependent Variable: Body Mass Index
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4
a Set to zero because this parameter is redundant.

Table 6e: Parameter Estimates for GEE model for MUAC change from 2 weeks to 9 months post delivery

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	21.383	1.5649	18.315	24.450	186.701	1	.000
[Group=2] Breastfeeding control	.011	.5454	-1.059	1.080	.000	1	.985
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.033	.0085	.016	.049	14.686	1	.000
[Group=2] * Timeinweeks	.019	.0120	-.005	.042	2.391	1	.122
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.246	.0571	.134	.357	18.486	1	.000
CD4	.001	.0002	.000	.001	15.884	1	.000
(Scale)	12.837						

Dependent Variable: Mid upper arm circumference

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4

a Set to zero because this parameter is redundant.

Table 6f: Parameter Estimates for GEE model for TSF change from 2 weeks to 9 months post delivery

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	10.782	2.7809	5.331	16.232	15.032	1	.000
[Group=2] Breastfeeding control	-.091	1.0062	-2.063	1.881	.008	1	.928
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.137	.0236	.091	.183	33.559	1	.000
[Group=2] * Timeinweeks	.032	.0348	-.036	.101	.864	1	.353
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.200	.0992	.005	.394	4.047	1	.044
CD4	.002	.0008	.001	.004	8.783	1	.003
(Scale)	40.840						

Dependent Variable: Triceps skin fold

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4

a Set to zero because this parameter is redundant.

Table 6g: Parameter Estimates for GEE model for LBM change from 2 weeks to 9 months post delivery

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	36.821	2.7055	31.519	42.124	185.231	1	.000
[Group=2] Breastfeeding control	.111	1.0421	-1.932	2.154	.011	1	.915
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	-.160	.0277	-.215	-.106	33.533	1	.000
[Group=2] * Timeinweeks	.005	.0397	-.073	.083	.014	1	.904
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.339	.0943	.154	.523	12.905	1	.000
CD4	-.001	.0013	-.003	.002	.417	1	.519
(Scale)	31.077						

Dependent Variable: Lean body mass

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4

a Set to zero because this parameter is redundant.

Table 6h: Parameter Estimates for GEE model for Fat mass change from 2 weeks to 9 months post delivery

Parameter	**B**	**Std. Error**	**95% Wald Confidence Interval**		**Hypothesis Test**		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	6.145	5.2137	-4.074	16.363	1.389	1	.239
[Group=2] Breastfeeding control	-.197	1.5151	-3.166	2.773	.017	1	.897
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.163	.0480	.068	.257	11.450	1	.001
[Group=2] * Timeinweeks	.019	.0579	-.095	.132	.102	1	.749
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.468	.1524	.169	.767	9.417	1	.002
CD4	.008	.0050	-.002	.017	2.439	1	.118
(Scale)	76.964						

Dependent Variable: Fat mass

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4

a Set to zero because this parameter is redundant.

Table 6i: Parameter Estimates for GEE model for Percentage fat mass change from 2 weeks to 9 months post delivery

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	22.815	4.1768	14.629	31.002	29.838	1	.000
[Group=2] Breastfeeding control	.053	1.4105	-2.711	2.818	.001	1	.970
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Time in weeks	.223	.0497	.126	.321	20.234	1	.000
[Group=2] * Time in weeks	.028	.0629	-.096	.151	.194	1	.659
[Group=1] * Time in weeks	0(a)	.	.	.	.	.	.
Baseline age	.256	.1399	-.018	.530	3.351	1	.067
CD4	.005	.0027	.000	.010	3.193	1	.074
(Scale)	60.264						

Dependent Variable: Percentage Fat mass
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4
a Set to zero because this parameter is redundant.

Appendix 11

GLM using Poisson distribution for comparing incidence rates of opportunistic infections and other events between the mothers who were in the breastfeeding control group and the formula feeding group

Table 9b: Parameter Estimates for a GLM model for the event of thrush over the follow-up period

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-39.610	2.1731	-43.869	-35.351	332.248	1	.000	6.28E-018	8.87E-020	4.44E-016
[Group=2] Breastfeeding control	-1.086	.8226	-2.698	.527	1.742	1	.187	.338	.067	1.693
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
CD4	-.002	.0022	-.007	.002	1.223	1	.269	.998	.993	1.002
Age	.092	.0591	-.023	.208	2.446	1	.118	1.097	.977	1.232
(Scale)	1(b)									

Dependent Variable: ORALTHRUSH_sum
Model: (Intercept), Group, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 9c: Parameter Estimates for a GLM model for the event of PID over the follow up period

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-34.981	1.6554	-38.225	-31.736	446.549	1	.000	6.43E-016	2.51E-017	1.65E-014
[Group=2] Breastfeeding control	-.584	.4740	-1.513	.345	1.517	1	.218	.558	.220	1.412
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
CD4	-.005	.0018	-.008	-.001	6.514	1	.011	.995	.992	.999
Age	-.020	.0470	-.112	.072	.175	1	.676	.981	.894	1.075
(Scale)	1(b)									

Dependent Variable: SignsofPID_sum
Model: (Intercept), Group, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 9d: Parameter Estimates for a GLM model for the event of LRTI over the follow up period

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-39.889	2.2663	-44.331	-35.447	309.786	1	.000	4.75E-018	5.59E-020	4.03E-016
[Group=2] Breastfeeding control	-.514	.7180	-1.921	.894	.512	1	.474	.598	.146	2.444
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
CD4	.002	.0016	-.001	.006	2.448	1	.118	1.002	.999	1.006
age	.012	.0680	-.121	.146	.034	1	.855	1.013	.886	1.157
(Scale)	1(b)									

Dependent Variable: SignsofLRTI_sum
Model: (Intercept), Group, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 9e: Parameter Estimates for a GLM model for the event of breast pathology over the follow up period

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-37.463	3.2077	-43.750	-31.176	136.397	1	.000	5.37E-017	1.00E-019	2.89E-014
[Group=2] Breastfeeding control	1.497	1.1313	-.720	3.714	1.751	1	.186	4.468	.487	41.029
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
CD4	-.004	.0036	-.011	.003	1.464	1	.226	.996	.989	1.003
age	-.027	.0913	-.206	.153	.084	1	.772	.974	.814	1.165
(Scale)	1(b)									

Dependent Variable: Breast_sum
Model: (Intercept), Group, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 9f: Parameter Estimates for a GLM model for the event of genital ulcers over the follow up period

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.743	.7235	-38.161	-35.325	2579.093	1	.000	1.10E-016	2.67E-017	4.56E-016
[Group=2] Breastfeeding control	.023	.2165	-.401	.447	.011	1	.915	1.023	.670	1.564
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
CD4	.001	.0005	.000	.002	1.622	1	.203	1.001	1.000	1.002
age	.008	.0217	-.034	.051	.146	1	.702	1.008	.966	1.052
(Scale)	1(b)									

Dependent Variable: genital_sum
Model: (Intercept), Group, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 9g: Parameter Estimates for a GLM model for the event of an SRQ20 Score ≥ 8 over the follow up period

Parameter	B		95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
		Std. Error	Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-37.418	1.3934	-40.149	-34.687	721.115	1	.000	5.62E-017	3.66E-018	8.62E-016
[Group=2] Breastfeeding control	-.982	.4852	-1.933	-.032	4.100	1	.043	.374	.145	.969
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
CD4	.000	.0012	-.003	.002	.122	1	.727	1.000	.997	1.002
age	.017	.0402	-.062	.095	.172	1	.679	1.017	.940	1.100
(Scale)	1(b)									

Dependent Variable: depsrq_sum
Model: (Intercept), Group, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Appendix 12

GEE models comparing anthropometric measures between breastfed control infants and the formula fed infants

Table 10d: Parameter Estimates for GEE model for weight change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	3.223	.7300	1.793	4.654	19.500	1	.000
[Group=2]Breastfeeding control	.278	.1175	.047	.508	5.583	1	.018
[Group=1]Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.158	.0047	.149	.167	1146.717	1	.000
[Group=2] * Timeinweeks	.006	.0070	-.007	.020	.794	1	.373
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-1.38E-005	.0001	.000	.000	.011	1	.918
MomBMI	.035	.0119	.012	.059	8.715	1	.003
Yearsofschool	.019	.0257	-.031	.069	.549	1	.459
Accesstowater	.245	.1336	-.017	.507	3.366	1	.067
Accesstoelectricity	-.160	.1985	-.549	.229	.648	1	.421
Sanitation	-.460	.1658	-.785	-.135	7.697	1	.006
(Scale)	.849						

Dependent Variable: weight2

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 10e: Parameter Estimates for GEE model for length change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	50.105	2.5487	45.109	55.100	386.467	1	.000
[Group=2]Breastfeeding control	.193	.3763	-.544	.931	.263	1	.608
[Group=1]Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.566	.0073	.552	.580	5996.800	1	.000
[Group=2] * Timeinweeks	.005	.0124	-.019	.029	.175	1	.676
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-3.19E-005	.0005	-.001	.001	.005	1	.945
MomBMI	.079	.0325	.015	.143	5.905	1	.015
Yearsofschool	.025	.0914	-.155	.204	.072	1	.788
Accesstowater	.765	.4385	-.095	1.624	3.041	1	.081
Accesstoelectricity	-.363	.6308	-1.599	.874	.331	1	.565
Sanitation	-1.164	.5453	-2.233	-.095	4.557	1	.033
(Scale)	6.192						

Dependent Variable: length

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation a Set to zero because this parameter is redundant.

Table 10f: Parameter Estimates for GEE model for head circumference change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	36.761	1.3194	34.175	39.347	776.332	1	.000
[Group=2]Breastfeeding control	.053	.2345	-.406	.513	.051	1	.821
[Group=1]Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.255	.0042	.247	.264	3645.384	1	.000
[Group=2] * Timeinweeks	-.002	.0078	-.017	.014	.042	1	.838
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	.000	.0002	.000	.001	.218	1	.640
MomBMI	.049	.0216	.007	.091	5.147	1	.023
Yearsofschool	.004	.0460	-.086	.095	.009	1	.923
Accesstowater	.176	.2463	-.306	.659	.512	1	.474
Accesstoelectricity	-.477	.3337	-1.131	.177	2.044	1	.153
Sanitation	-.577	.2891	-1.144	-.011	3.988	1	.046
(Scale)	2.516						

Dependent Variable: hc

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 10g: Parameter Estimates for GEE model for MUAC change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
	Lower	Upper			Wald Chi-Square	df	Sig.
(Intercept)	9.998	1.0226	7.993	12.002	95.584	1	.000
[Group=2]	.548	.1656	.224	.873	10.966	1	.001
[Group=1]	0(a)	.	.	.	.	.	.
Timeinweeks	.127	.0062	.115	.139	419.954	1	.000
[Group=2] * Timeinweeks	-.001	.0085	-.018	.015	.020	1	.887
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	.000	.0002	-.001	.000	1.063	1	.302
MomBMI	.047	.0145	.019	.076	10.650	1	.001
Yearsofschool	.017	.0320	-.045	.080	.298	1	.585
Accesstowater	.198	.1831	-.161	.557	1.168	1	.280
Accesstoelectricity	-.083	.2826	-.637	.471	.086	1	.769
Sanitation	-.367	.2301	-.818	.084	2.551	1	.110
(Scale)	1.599						

Dependent Variable: ac

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 10h:Parameter Estimates for GEE model for TSF change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	6.978	1.6242	3.795	10.162	18.459	1	.000
[Group=2]Breastfeeding control	.847	.2568	.344	1.351	10.889	1	.001
[Group=1]Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.112	.0095	.094	.131	138.361	1	.000
[Group=2] * Timeinweeks	-.005	.0136	-.032	.021	.149	1	.699
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	.000	.0004	-.001	.001	.301	1	.583
MomBMI	.036	.0229	-.009	.081	2.462	1	.117
Yearsofschool	.069	.0670	-.062	.200	1.060	1	.303
Accesstowater	.261	.2926	-.313	.834	.793	1	.373
Accesstoelectricity	-.314	.4365	-1.170	.541	.519	1	.471
Sanitation	-.652	.3632	-1.364	.060	3.221	1	.073
(Scale)	4.146						

Dependent Variable: ts

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 10i: Parameter Estimates for GEE model for BMI change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	14.316	1.3418	11.686	16.946	113.840	1	.000
[Group=2]Breastfeeding control	.796	.2532	.300	1.293	9.891	1	.002
[Group=1]Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.118	.0083	.102	.134	200.614	1	.000
[Group=2] * Timeinweeks	.002	.0125	-.023	.026	.016	1	.899
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	3.62E-005	.0003	-.001	.001	.011	1	.915
MomBMI	.048	.0230	.003	.093	4.411	1	.036
Yearsofschool	.035	.0596	-.082	.152	.339	1	.560
Accesstowater	.239	.2587	-.268	.746	.853	1	.356
Accesstoelectricity	-.308	.3599	-1.014	.397	.734	1	.392
Sanitation	-.593	.3121	-1.205	.018	3.615	1	.057
(Scale)	3.467						

Dependent Variable: BMI

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 10j: Parameter Estimates for GEE model for z score for weight for age change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-.812	.8927	-2.562	.938	.828	1	.363
[Group=2]Breastfeeding control	.411	.1712	.076	.747	5.767	1	.016
[Group=1]Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.021	.0046	.012	.030	19.947	1	.000
[Group=2] * Timeinweeks	.001	.0074	-.013	.016	.023	1	.879
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	.000	.0002	.000	.000	.455	1	.500
MomBMI	.038	.0144	.009	.066	6.769	1	.009
Yearsofschool	.037	.0315	-.025	.099	1.367	1	.242
Accesstowater	.198	.1615	-.118	.515	1.505	1	.220
Accesstoelectricity	-.367	.2324	-.822	.089	2.487	1	.115
Sanitation	-.488	.2005	-.880	-.095	5.916	1	.015
(Scale)	1.037						

Dependent Variable: zwaz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 10k: Parameter Estimates for GEE model for z score for length for age change over time in infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-.981	1.0781	-3.094	1.132	.828	1	.363
[Group=2]Breastfeeding control	.078	.1754	-.265	.422	.200	1	.655
[Group=1]Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.011	.0033	.004	.017	10.954	1	.001
[Group=2] * Timeinweeks	.000	.0052	-.010	.010	.001	1	.973
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	9.10E-005	.0002	.000	.000	.238	1	.625
MomBMI	.029	.0141	.001	.056	4.209	1	.040
Yearsofschool	.025	.0462	-.066	.115	.287	1	.592
Accesstowater	.222	.1855	-.141	.586	1.434	1	.231
Accesstoelectricity	-.339	.2565	-.842	.163	1.750	1	.186
Sanitation	-.429	.2272	-.874	.016	3.568	1	.059
(Scale)	1.018						

Dependent Variable: zhaz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 10l: Parameter Estimates for GEE model for z score for head circumference for age change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	9.494	8.7277	-7.612	26.600	1.183	1	.277
[Group=2] Breastfeeding control	-4.416	4.4568	-13.152	4.319	.982	1	.322
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.001	.0062	-.011	.013	.031	1	.860
[Group=2] * Timeinweeks	.403	.3989	-.379	1.185	1.022	1	.312
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	.001	.0008	-.001	.002	.843	1	.359
MomBMI	-.139	.1657	-.464	.185	.707	1	.401
Yearsofschool	-.159	.2126	-.576	.258	.557	1	.455
Accesstowater	1.491	1.4437	-1.339	4.320	1.066	1	.302
Accesstoelectricity	-2.635	2.0328	-6.619	1.350	1.680	1	.195
Sanitation	-1.171	.9401	-3.014	.672	1.551	1	.213
(Scale)	1041.277						

Dependent Variable: zhcz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 10m: Parameter Estimates for GEE model for z score for MUAC for age change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-1.052	1.1207	-3.249	1.144	.881	1	.348
[Group=2] Breastfeeding control	.865	.2350	.404	1.325	13.544	1	.000
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.027	.0065	.015	.040	17.542	1	.000
[Group=2] * Timeinweeks	-.017	.0084	-.034	-.001	4.164	1	.041
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	.000	.0002	-.001	.000	.873	1	.350
MomBMI	.041	.0168	.008	.074	6.079	1	.014
Yearsofschool	.028	.0469	-.064	.120	.354	1	.552
Accesstowater	.101	.2009	-.292	.495	.254	1	.614
Accesstoelectricity	-.194	.2959	-.774	.386	.431	1	.511
Sanitation	-.429	.2453	-.910	.052	3.061	1	.080
(Scale)	1.133						

Dependent Variable: zacz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 10n: Parameter Estimates for GEE model for z score for TSF for age change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-.359	1.2203	-2.751	2.032	.087	1	.768
[Group=2]	.691	.2271	.245	1.136	9.244	1	.002
[Group=1]	0(a)	.	.	.	.	.	.
Timeinweeks	.031	.0058	.020	.043	28.905	1	.000
[Group=2] * Timeinweeks	-.012	.0077	-.027	.003	2.264	1	.132
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-7.94E-005	.0002	.000	.000	.146	1	.702
MomBMI	.019	.0151	-.011	.049	1.578	1	.209
Yearsofschool	.051	.0705	-.088	.189	.513	1	.474
Accesstowater	.091	.1860	-.274	.455	.238	1	.625
Accesstoelectricity	-.206	.2853	-.765	.353	.523	1	.470
Sanitation	-.469	.2444	-.949	.010	3.688	1	.055
(Scale)	1.009						

Dependent Variable: ztsz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 10o: Parameter Estimates for GEE model for z score for BMI for age change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-.396	.8818	-2.124	1.332	.202	1	.653
[Group=2]Breastfeeding control	.526	.1681	.196	.855	9.770	1	.002
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.020	.0052	.010	.030	14.821	1	.000
[Group=2] * Timeinweeks	.000	.0079	-.015	.016	.000	1	.983
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	9.52E-005	.0002	.000	.001	.211	1	.646
MomBMI	.030	.0152	.000	.059	3.771	1	.052
Yearsofschool	.030	.0372	-.043	.103	.665	1	.415
Accesstowater	.111	.1626	-.208	.430	.467	1	.494
Accesstoelectricity	-.235	.2292	-.685	.214	1.054	1	.305
Sanitation	-.335	.1991	-.725	.056	2.824	1	.093
(Scale)	1.139						

Dependent Variable: zBMI

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 10p: Parameter Estimates for GEE model for z score for weight for length change over time in the infant

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-.098	.8905	-1.843	1.647	.012	1	.912
[Group=2]Breastfeeding control	.516	.1679	.187	.846	9.459	1	.002
[Group=1]Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.014	.0053	.003	.024	6.762	1	.009
[Group=2] * Timeinweeks	-.001	.0077	-.016	.014	.015	1	.902
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	3.90E-005	.0002	.000	.000	.028	1	.868
MomBMI	.020	.0151	-.010	.049	1.735	1	.188
Yearsofschool	.021	.0420	-.061	.103	.249	1	.618
Accesstowater	.056	.1643	-.266	.378	.116	1	.733
Accesstoelectricity	-.087	.2216	-.521	.347	.154	1	.695
Sanitation	-.189	.1895	-.560	.183	.993	1	.319
(Scale)	1.165						

Dependent Variable: zwhz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Yearsofschool, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Appendix 13

GLM with Poisson distribution comparing incidence rates of opportunistic infections between breastfed control infants and the formula fed infants

Table 11d: Parameter Estimates for the GLM model for the event of diarrhoea in the infant at 14 weeks of age

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig		Lower	Upper
(Intercept)	-15.169	.2085	-15.577	-14.760	5292.140	1	.000	2.58E-007	1.72E-007	3.89E-007
[Group=2] Breastfeeding control	-1.183	.4584	-2.081	-.284	6.655	1	.010	.306	.125	.753
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: Hodiarrhoea_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 11e: Parameter Estimates for the GLM model for the event of diarrhoea in the infant in the follow-up time

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.220	.1325	-36.479	-35.960	74776.111	1	.000	1.86E-016	1.44E-016	2.41E-016
[Group=2]Breastfeeding control	-.361	.2187	-.790	.067	2.730	1	.098	.697	.454	1.070
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: diarrhoea_sum
Model: (Intercept), Group, offset = Follow up time weeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 11f: Parameter Estimates for the GLM model for the event of ear discharge in the infant in the followup time

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-38.317	.3780	-39.058	-37.576	10277.225	1	.000	2.29E-017	1.09E-017	4.80E-017
[Group=2]Breastfeeding control	-.151	.5855	-1.299	.996	.067	1	.796	.860	.273	2.708
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: Hoeardischarge_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 11g: Parameter Estimates for the GLM model for the event of LRTI in the infant in the followup time

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-39.570	.7071	-40.955	-38.184	3131.496	1	.000	6.53E-018	1.63E-018	2.61E-017
[Group=2] Breastfeeding control	.878	.8660	-.819	2.576	1.029	1	.311	2.407	.441	13.140
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: SignsofLRTI_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 11h: Parameter Estimates for the GLM model for the event of failure to thrive in the infant during the entire follow-up

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig		Lower	Upper
(Intercept)	-40.263	1.0000	-42.223	-38.303	1621.084	1	.000	3.27E-018	4.60E-019	2.32E-017
[Group=2] Breastfeeding control	.878	1.2247	-1.522	3.279	.514	1	.473	2.407	.218	26.542
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: ftt_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 11i: Parameter Estimates for the GLM model for the event of PTB in the infant in the entire follow-up period

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-39.164	.5774	-40.296	-38.032	4601.473	1	.000	9.80E-018	3.16E-018	3.04E-017
[Group=2] Breastfeeding control	1.032	.6901	-.320	2.385	2.238	1	.135	2.808	.726	10.858
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: tb_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 11j: Parameter Estimates for the GLM model for the event of skin sepsis in the infant in the entire follow-up period

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-38.065	.3333	-38.719	-37.412	13040.811	1	.000	2.94E-017	1.53E-017	5.65E-017
[Group=2] Breastfeeding control	.185	.4714	-.739	1.109	.154	1	.695	1.203	.478	3.032
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: skinsepsis_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 11k: Parameter Estimates for the GLM model for the event of meningitis in the infant in the entire follow-up period

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-40.263	1.0000	-42.223	-38.303	1621.083	1	.000	3.27E-018	4.60E-019	2.32E-017
[Group=2] Breastfeeding control	.185	1.4142	-2.587	2.957	.017	1	.896	1.203	.075	19.239
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: meningitis_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 11l: Parameter Estimates for the GLM model for the event of delayed milestones in the infant in the entire follow-up period

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-38.065	.3333	-38.719	-37.412	13040.811	1	.000	2.94E-017	1.53E-017	5.65E-017
[Group=2] Breastfeeding control	-.220	.5270	-1.253	.813	.175	1	.676	.802	.286	2.254
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: development_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 11m: Parameter Estimates for the GLM model for the event of death in the infant in the entire follow-up period

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square.	Df	Sig		Lower	Upper
(Intercept)	-39.570	.7071	-40.955	-38.184	3131.496	1	.000	6.53E-018	1.63E-018	2.61E-017
[Group=2]Breastfeeding control	.185	1.0000	-1.775	2.145	.034	1	.853	1.203	.170	8.543
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: Child status
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Appendix 14

GEE models using exchangeable covariance matrix comparing anthropometric measures between HIV infected breastfed control and formula fed infants

Table 12b: Parameter Estimates of a GEE model for weight over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square.	Df	Sig
(Intercept)	10.144	7.6672	-4.883	25.172	1.750	1	.186
[Group=2] Breastfeeding control	2.625	3.4236	-4.085	9.335	.588	1	.443
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.136	.0073	.121	.150	347.343	1	.000
[Group=2] * Timeinweeks	-.011	.0223	-.055	.033	.249	1	.618
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-.008	.0051	-.018	.002	2.665	1	.103
MomBMI	.225	.3631	-.487	.936	.382	1	.536
Accesstowater	-.637	3.0680	-6.650	5.376	.043	1	.835
Accesstoelectricity	-4.848	3.4424	-11.595	1.899	1.983	1	.159
Sanitation	-1.662	2.3532	-6.275	2.950	.499	1	.480
(Scale)	.563						

Dependent Variable: weight2
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation
a Set to zero because this parameter is redundant.

Table 12c: Parameter Estimates of a GEE model for length over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	92.222	5.8048	80.844	103.599	252.400	1	.000
[Group=2] Breastfeeding control	-7.350	4.0656	-15.318	.619	3.268	1	.071
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.503	.0340	.436	.570	218.737	1	.000
[Group=2] * Timeinweeks	.013	.0477	-.080	.107	.075	1	.784
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-.013	.0045	-.022	-.004	8.282	1	.004
MomBMI	-1.024	.4066	-1.820	-.227	6.336	1	.012
Accesstowater	6.861	3.3604	.275	13.447	4.169	1	.041
Accesstoelectricity	-1.914	3.8140	-9.389	5.561	.252	1	.616
Sanitation	-7.666	1.7366	-11.070	-4.263	19.488	1	.000
(Scale)	2.496						

Dependent Variable: length
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation
a Set to zero because this parameter is redundant.

Table 12d: Parameter Estimates of a GEE model for head circumference over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	53.784	14.2582	25.838	81.729	14.229	1	.000
[Group=2] Breastfeeding control	5.377	6.4888	-7.341	18.095	.687	1	.407
[Group=1]Formula feeding	0(a)	.	.	.	.	.	.
Time in weeks	.237	.0170	.204	.271	193.848	1	.000
[Group=2] * Time in weeks	-.002	.0294	-.059	.056	.003	1	.953
[Group=1] * Time in weeks	0(a)	.	.	.	.	.	.
MomCD4	-.019	.0099	-.038	.001	3.598	1	.058
MomBMI	.558	.6878	-.790	1.906	.658	1	.417
Accesstowater	.105	5.7125	-11.091	11.302	.000	1	.985
Accesstoelectricity	-11.612	6.6743	-24.693	1.470	3.027	1	.082
Sanitation	-7.126	4.3880	-15.726	1.475	2.637	1	.104
(Scale)	1.408						

Dependent Variable: hc
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation
a Set to zero because this parameter is redundant.

Table 12e: Parameter Estimates of a GEE model for MUAC over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig
(Intercept)	11.022	7.2023	-3.094	25.138	2.342	1	.126
[Group=2]Breastfeeding control	5.030	4.0082	-2.825	12.886	1.575	1	.209
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.093	.0266	.041	.145	12.122	1	.000
[Group=2] * Timeinweeks	-.016	.0368	-.088	.056	.190	1	.663
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-.010	.0054	-.020	.001	3.129	1	.077
MomBMI	.459	.4119	-.348	1.266	1.242	1	.265
Accesstowater	-3.129	3.3717	-9.738	3.479	.861	1	.353
Accesstoelectricity	-5.624	4.0121	-13.488	2.240	1.965	1	.161
Sanitation	1.087	2.2027	-3.230	5.404	.244	1	.622
(Scale)	.985						

Dependent Variable: ac
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation
a Set to zero because this parameter is redundant.

Table 12f: Parameter Estimates of a GEE model for TSF over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-15.102	3.4247	-21.814	-8.390	19.446	1	.000
[Group=2] Breastfeeding control	16.754	3.2367	10.410	23.098	26.792	1	.000
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.104	.0424	.021	.188	6.074	1	.014
[Group=2] * Timeinweeks	-.070	.0485	-.165	.025	2.073	1	.150
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-.010	.0036	-.017	-.003	8.536	1	.003
MomBMI	1.577	.3401	.910	2.243	21.491	1	.000
Accesstowater	-14.453	2.7450	-19.833	-9.073	27.722	1	.000
Accesstoelectricity	-11.999	3.2921	-18.451	-5.547	13.285	1	.000
Sanitation	8.884	1.0007	6.923	10.845	78.819	1	.000
(Scale)	1.916						

Dependent Variable: ts
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation
a Set to zero because this parameter is redundant.

Table 12g: Parameter Estimates of a GEE model for BMI over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	11.837	18.5348	-24.491	48.164	.408	1	.523
[Group=2] Breastfeeding control	8.242	8.3279	-8.080	24.564	.979	1	.322
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.121	.0436	.036	.207	7.714	1	.005
[Group=2] * Timeinweeks	-.047	.0495	-.144	.050	.908	1	.341
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-.012	.0126	-.037	.013	.916	1	.339
MomBMI	.815	.8707	-.892	2.521	.875	1	.350
Accesstowater	-3.312	7.2945	-17.609	10.985	.206	1	.650
Accesstoelectricity	-8.873	8.3905	-25.318	7.572	1.118	1	.290
Sanitation	-.541	5.7058	-11.724	10.642	.009	1	.924
(Scale)	2.595						

Dependent Variable: BMI
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation
a Set to zero because this parameter is redundant.

Table 12h: Parameter Estimates of a GEE model for z score for weight for age over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-1.464	10.9262	-22.879	19.951	.018	1	.893
[Group=2] Breastfeeding control	3.670	4.9267	-5.986	13.326	.555	1	.456
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	-.001	.0138	-.028	.026	.007	1	.931
[Group=2] * Timeinweeks	-.018	.0235	-.064	.028	.615	1	.433
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-.003	.0074	-.018	.011	.225	1	.635
MomBMI	.282	.5156	-.729	1.292	.299	1	.585
Accesstowater	-1.603	4.3301	-10.090	6.884	.137	1	.711
Accesstoelectricity	-4.013	4.9359	-13.688	5.661	.661	1	.416
Sanitation	.585	3.3602	-6.001	7.171	.030	1	.862
(Scale)	.918						

Dependent Variable: zwaz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 12i: Parameter Estimates of a GEE model for z score for length for age over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig
(Intercept)	5.293	2.4557	.480	10.106	4.646	1	.031
[Group=2] Breastfeeding control	-1.462	1.6454	-4.687	1.763	.789	1	.374
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	-.023	.0112	-.045	-.001	4.350	1	.037
[Group=2] * Timeinweeks	.016	.0167	-.016	.049	.945	1	.331
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	.001	.0019	-.003	.005	.273	1	.602
MomBMI	-.268	.1633	-.588	.052	2.691	1	.101
Accesstowater	1.004	1.3499	-1.642	3.650	.553	1	.457
Accesstoelectricity	-.020	1.5399	-3.038	2.999	.000	1	.990
Sanitation	-.078	.7392	-1.527	1.371	.011	1	.916
(Scale)	.343						

Dependent Variable: zhaz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 12j: Parameter Estimates of a GEE model for z score for BMI for age over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-6.425	12.7859	-31.485	18.635	.253	1	.615
[Group=2] Breastfeeding control	5.777	5.7016	-5.398	16.952	1.027	1	.311
[Group=1]Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.022	.0292	-.036	.079	.546	1	.460
[Group=2] * Timeinweeks	-.037	.0333	-.102	.029	1.214	1	.270
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-.005	.0087	-.022	.012	.317	1	.573
MomBMI	.554	.5970	-.616	1.724	.862	1	.353
Accesstowater	-2.707	4.9987	-12.504	7.091	.293	1	.588
Accesstoelectricity	-4.966	5.7631	-16.262	6.329	.743	1	.389
Sanitation	.957	3.9380	-6.762	8.675	.059	1	.808
(Scale)	1.140						

Dependent Variable: zBMI
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation
a Set to zero because this parameter is redundant.

Table 12k: Parameter Estimates of a GEE model for z score for weight for length over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-8.574	11.5029	-31.119	13.971	.556	1	.456
[Group=2] Breastfeeding control	6.995	4.9709	-2.748	16.737	1.980	1	.159
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.010	.0215	-.032	.052	.205	1	.651
[Group=2] * Timeinweeks	-.038	.0277	-.093	.016	1.924	1	.165
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-.006	.0077	-.021	.009	.629	1	.428
MomBMI	.734	.5285	-.301	1.770	1.931	1	.165
Accesstowater	-3.631	4.4408	-12.334	5.073	.668	1	.414
Accesstoelectricity	-5.828	5.0908	-15.806	4.150	1.311	1	.252
Sanitation	1.128	3.5426	-5.815	8.072	.101	1	.750
(Scale)	.888						

Dependent Variable: zwhz
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation
a Set to zero because this parameter is redundant.

Table 12l: Parameter Estimates of a GEE model for z score for MUAC for age over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-3.257	8.6342	-20.179	13.666	.142	1	.706
[Group=2] Breastfeeding control	10.492	5.3119	.081	20.903	3.901	1	.048
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.058	.0487	-.038	.153	1.408	1	.235
[Group=2] * Timeinweeks	-.082	.0560	-.192	.027	2.159	1	.142
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-.013	.0067	-.026	.000	4.024	1	.045
MomBMI	.878	.5198	-.141	1.896	2.850	1	.091
Accesstowater	-5.513	4.2405	-13.825	2.798	1.690	1	.194
Accesstoelectricity	-10.054	5.0725	-19.996	-.112	3.929	1	.047
Sanitation	.489	2.6323	-4.670	5.648	.034	1	.853
(Scale)	.861						

Dependent Variable: zacz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 12m: Parameter Estimates of a GEE model for z score for TSF for age over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-20.705	.6965	-22.070	-19.340	883.611	1	.000
[Group=2] Breastfeeding control	11.605	1.1184	9.413	13.797	107.675	1	.000
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	.062	.0464	-.029	.153	1.766	1	.184
[Group=2] * Timeinweeks	-.063	.0479	-.157	.031	1.748	1	.186
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-.004	.0004	-.005	-.004	126.856	1	.000
MomBMI	1.099	.0466	1.008	1.191	556.064	1	.000
Accesstowater	-9.485	.3790	-10.228	-8.742	626.212	1	.000
Accesstoelectricity	-7.229	.4596	-8.130	-6.328	247.461	1	.000
Sanitation	6.591	.1639	6.269	6.912	1617.012	1	.000
(Scale)	.389						

Dependent Variable: ztsz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Table 12n: Parameter Estimates of a GEE model for z score for head circumference for age over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square.	Df	Sig
(Intercept)	1.058	11.5706	-21.620	23.736	.008	1	.927
[Group=2]Breastfeeding control	7.222	5.1409	-2.854	17.298	1.973	1	.160
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.
Timeinweeks	-.019	.0128	-.044	.006	2.189	1	.139
[Group=2] * Timeinweeks	.007	.0216	-.035	.050	.115	1	.734
[Group=1] * Timeinweeks	0(a)	.	.	.	.	.	.
MomCD4	-.009	.0079	-.025	.006	1.380	1	.240
MomBMI	.755	.5468	-.317	1.827	1.906	1	.167
Accesstowater	-2.822	4.5506	-11.741	6.097	.385	1	.535
Accesstoelectricity	-9.534	5.3020	-19.926	.858	3.233	1	.072
Sanitation	-2.294	3.5633	-9.278	4.690	.414	1	.520
(Scale)	.763						

Dependent Variable: zhcz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, MomCD4, MomBMI, Accesstowater, Accesstoelectricity, Sanitation

a Set to zero because this parameter is redundant.

Appendix 15

GLM using Poisson distribution comparing incidence rates of opportunistic infections amongst the HIV infected breastfed and formula fed infants

Table 13c: **Parameter Estimates of GLM for the event of oral thrush amongst the infected infants**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-37.099	1.0000	-39.059	-35.139	1376.307	1	.000	7.73E-017	1.09E-017	5.49E-016
[Group=2] Breastfeeding control	1.099	1.0954	-1.048	3.246	1.006	1	.316	3.000	.350	25.678
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: ORALTHRUSH_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 13d: **Parameter Estimates of GLM for the event of diarrhoea amongst the infected infants**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.000	.5774	-37.132	-34.868	3888.000	1	.000	2.32E-016	7.48E-017	7.19E-016
[Group=2] Breastfeeding control	-3.56E-011	.7303	-1.431	1.431	.000	1	1.000	1.000	.239	4.184
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: Hodiarrhoea_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 13e: **Parameter Estimates of GLM for the event of ear discharge amongst the infected infants**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-37.099	1.0000	-39.059	-35.139	1376.307	1	.000	7.73E-017	1.09E-017	5.49E-016
[Group=2]Breastfeeding control	.182	1.2247	-2.218	2.583	.022	1	.882	1.200	.109	13.234
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: Hoeardischarge_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 13f: **Parameter Estimates of GLM for the event of LRTI amongst the infected infants**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-37.099	1.0000	-39.059	-35.139	1376.307	1	.000	7.73E-017	1.09E-017	5.49E-016
[Group=2] Breastfeeding control	.588	1.1547	-1.675	2.851	.259	1	.611	1.800	.187	17.304
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: SignsofLRTI_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 13g: **Parameter Estimates of GLM for the event of FTT amongst the infected infants**

Parameter	B(a)	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-64.307	.7071	-65.693	-62.921	8270.735	1	.000	1.18E-028	2.95E-029	4.72E-028
[Group=2] Breastfeeding control	.000	.	.	.	.	.	.	1.000	.000	.000
[Group=1] Formula feeding	0(b)	.	.	.	.	.	.	1	.	.
(Scale)	1©									

Dependent Variable: ftt_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Some parameter estimates are set to zero because the Hessian matrix is singular.
B Set to zero because this parameter is redundant.
C Fixed at the displayed value.

Table 13h: **Parameter Estimates of GLM for the event of PTB amongst the infected infants**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-37.099	1.0000	-39.059	-35.139	1376.307	1	.000	7.73E-017	1.09E-017	5.49E-016
[Group=2] Breastfeeding control	1.099	1.0954	-1.048	3.246	1.006	1	.316	3.000	.350	25.678
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: tb_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 13i: **Parameter Estimates of GLM for the event of meningitis amongst the infected infants**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-37.099	1.0000	-39.059	-35.139	1376.307	1	.000	7.73E-017	1.09E-017	5.49E-016
[Group=2] Breastfeeding control	-.511	1.4142	-3.283	2.261	.130	1	.718	.600	.038	9.593
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: meningitis_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 13j: **Parameter Estimates of GLM for the event of delay milestones amongst the infected infants**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-34.901	.3333	-35.555	-34.248	10962.962	1	.000	6.96E-016	3.62E-016	1.34E-015
[Group=2] Breastfeeding control	-2.015	.7817	-3.547	-.483	6.643	1	.010	.133	.029	.617
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: development_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 13k: **Parameter Estimates of GLM for the event of hospital admissions amongst the infected infants**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.405	.7071	-37.791	-35.020	2650.716	1	.000	1.55E-016	3.87E-017	6.18E-016
[Group=2] Breastfeeding control	-.511	1.0000	-2.471	1.449	.261	1	.609	.600	.085	4.259
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: HOAdmissions_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Table 13l: **Parameter Estimates of GLM for the event of death amongst the infected infants**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-37.099	1.0000	-39.059	-35.139	1376.307	1	.000	7.73E-017	1.09E-017	5.49E-016
[Group=2] Breastfeeding control	.182	1.2247	-2.218	2.583	.022	1	.882	1.200	.109	13.234
[Group=1] Formula feeding	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: Child status
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
B Fixed at the displayed value.

Appendix 16

GEE models using exchangeable covariance matrix comparing body composition and anthropometric measures between the two breastfeeding groups

BMI>25 kg/m^2

Table 16e: Parameter Estimates for a GEE model for weight change over time in breastfeeding mothers with high BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	68.916	5.0222	59.073	78.759	188.302	1	.000
[Group=3] Breastfeeding supplemented	2.253	2.0487	-1.762	6.268	1.210	1	.271
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	-.038	.0350	-.106	.031	1.159	1	.282
[Group=3] * Timeinweeks	-.029	.0571	-.140	.083	.250	1	.617
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.151	.1803	-.202	.505	.705	1	.401
breastfeedingduration	-3.957	1.9004	-7.682	-.232	4.336	1	.037
CD4	.000	.0027	-.006	.005	.015	1	.901
(Scale)	79.773						

Dependent Variable: Weight

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, breastfeedingduration, CD4

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16f: Parameter Estimates for a GEE model for BMI change over time in breastfeeding mothers with high BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	26.435	2.0817	22.355	30.515	161.266	1	.000
[Group=3] Breastfeeding supplemented	1.006	.8578	-.675	2.688	1.376	1	.241
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	-.016	.0143	-.044	.012	1.261	1	.261
[Group=3] * Timeinweeks	-.011	.0232	-.056	.035	.216	1	.642
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.129	.0775	-.023	.281	2.778	1	.096
CD4	.000	.0011	-.002	.002	.027	1	.870
breastfeedingduration	-.982	.7865	-2.524	.559	1.560	1	.212
(Scale)	13.663						

Dependent Variable: Body Mass Index

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16g: Parameter Estimates for a GEE model for MUAC over time in breastfeeding mothers with high BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	27.623	1.6704	24.349	30.897	273.475	1	.000
[Group=3]Breastfeeding supplemented	.521	.5560	-.569	1.611	.878	1	.349
[Group=2]Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.043	.0116	.020	.065	13.555	1	.000
[Group=3] * Timeinweeks	.013	.0200	-.026	.052	.403	1	.525
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.084	.0623	-.038	.207	1.838	1	.175
CD4	-7.90E-005	.0008	-.002	.001	.011	1	.918
breastfeedingduration	-.691	.6101	-1.887	.505	1.282	1	.257
(Scale)	7.490						

Dependent Variable: Mid upper arm circumference

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16h: Parameter Estimates for a GEE model for TSF over time in breastfeeding mothers with high BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
	Lower	Upper			Wald Chi-Square	df	Sig.
(Intercept)	23.088	2.7220	17.753	28.423	71.940	1	.000
[Group=3] Breastfeeding supplemented	1.631	1.1495	-.622	3.884	2.013	1	.156
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.154	.0359	.084	.225	18.467	1	.000
[Group=3] * Timeinweeks	-.026	.0541	-.132	.081	.223	1	.637
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
Baselineage	-.167	.1077	-.378	.044	2.411	1	.120
CD4	.001	.0013	-.002	.003	.328	1	.567
breastfeedingduration	-2.933	1.1948	-5.275	-.591	6.025	1	.014
(Scale)	27.985						

Dependent Variable: Triceps skin fold

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16i: Parameter Estimates for a GEE model for LBM over time in breastfeeding mothers with high BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
	Lower	Upper			Wald Chi-Square	df	Sig.
(Intercept)	45.638	3.3918	38.990	52.286	181.046	1	.000
[Group=3] Breastfeeding supplemented	.551	1.3592	-2.113	3.215	.165	1	.685
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	-.155	.0478	-.248	-.061	10.452	1	.001
[Group=3] * Timeinweeks	-.016	.0580	-.130	.098	.077	1	.781
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.097	.1153	-.129	.323	.704	1	.401
CD4	-.003	.0013	-.005	.000	4.278	1	.039
breastfeedingduration	-.269	1.1322	-2.488	1.950	.056	1	.812
(Scale)	26.776						

Dependent Variable: Lean body mass

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16j: Parameter Estimates for a GEE model for Fat mass over time in breastfeeding mothers with high BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
	Lower	Upper	Wald Chi-Square	df	Sig.	Lower	Upper
(Intercept)	20.890	4.0461	12.960	28.821	26.657	1	.000
[Group=3] Breastfeeding supplemented	.733	1.7084	-2.616	4.081	.184	1	.668
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.135	.0489	.039	.231	7.597	1	.006
[Group=3] * Timeinweeks	.050	.0777	-.102	.203	.418	1	.518
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.145	.1482	-.145	.436	.963	1	.326
CD4	.003	.0020	-.001	.007	2.014	1	.156
breastfeedingduration	-4.421	1.6714	-7.697	-1.145	6.998	1	.008
(Scale)	45.848						

Dependent Variable: Fat mass

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16k: Parameter Estimates for a GEE model for percentage fat mass over time in breastfeeding mothers with high BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	30.862	4.0238	22.975	38.748	58.825	1	.000
[Group=3] Breastfeeding supplemented	.231	1.7380	-3.176	3.637	.018	1	.894
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.166	.0514	.066	.267	10.487	1	.001
[Group=3] * Timeinweeks	.045	.0806	-.113	.203	.309	1	.578
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
Baselineage	.114	.1422	-.165	.393	.643	1	.423
CD4	.004	.0019	2.68E-005	.007	3.897	1	.048
breastfeedingduration	-3.719	1.6823	-7.016	-.422	4.887	1	.027
(Scale)	40.736						

Dependent Variable: Percentage Fat mass

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

BMI≤24.9 kg/m^2

Table 16l: Parameter Estimates for a GEE model for weight change over time in breastfeeding mothers with low to normal BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	53.125	3.6164	46.037	60.212	215.799	1	.000
[Group=3] Breastfeeding supplemented	1.142	1.3329	-1.470	3.754	.734	1	.392
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.013	.0384	-.062	.089	.123	1	.726
[Group=3] * Timeinweeks	-.050	.0493	-.147	.046	1.036	1	.309
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
Baselineage	.261	.1256	.014	.507	4.306	1	.038
CD4	-.004	.0027	-.009	.001	2.310	1	.129
breastfeedingduration	-1.665	1.8180	-5.228	1.899	.838	1	.360
(Scale)	34.870						

Dependent Variable: Weight

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16m: Parameter Estimates for a GEE model for BMI over time in breastfeeding mothers with low to normal BMI at baseline

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
	Lower	Upper	Wald Chi-Square	df	Sig.	Lower	Upper
(Intercept)	19.550	1.2133	17.172	21.928	259.628	1	.000
[Group=3] Breastfeeding supplemented	.132	.3602	-.574	.838	.135	1	.713
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.010	.0153	-.020	.040	.398	1	.528
[Group=3] * Timeinweeks	-.024	.0194	-.062	.014	1.586	1	.208
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.144	.0451	.056	.233	10.237	1	.001
CD4	.000	.0009	-.002	.001	.089	1	.765
breastfeedingduration	-.311	.4909	-1.273	.652	.400	1	.527
(Scale)	2.939						

Dependent Variable: Body Mass Index

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16n: Parameter Estimates for a GEE model for MUAC in breastfeeding mothers with low to normal BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig
(Intercept)	22.468	1.2227	20.071	24.865	337.651	1	.000
[Group=3] Breastfeeding supplemented	.536	.4127	-.273	1.345	1.688	1	.194
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.061	.0140	.033	.088	18.802	1	.000
[Group=3] * Timeinweeks	-.032	.0177	-.066	.003	3.211	1	.073
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.120	.0416	.039	.202	8.344	1	.004
CD4	.000	.0008	-.002	.001	.096	1	.757
breastfeedingduration	-.790	.5294	-1.828	.247	2.228	1	.136
(Scale)	3.309						

Dependent Variable: Mid upper arm circumference

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16o: Parameter Estimates for a GEE model for TSF in breastfeeding mothers with low to normal BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	16.106	2.9032	10.416	21.796	30.777	1	.000
[Group=3] Breastfeeding supplemented	.257	.8060	-1.323	1.836	.101	1	.750
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.186	.0359	.116	.257	27.046	1	.000
[Group=3] * Timeinweeks	-.051	.0506	-.150	.049	.997	1	.318
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	-.064	.0983	-.257	.128	.427	1	.514
CD4	-.002	.0020	-.006	.002	.759	1	.384
breastfeedingduration	-1.825	1.0402	-3.864	.214	3.077	1	.079
(Scale)	16.027						

Dependent Variable: Triceps skin fold

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16p: Parameter Estimates for a GEE model for LBM in breastfeeding mothers with low to normal BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig
(Intercept)	35.610	3.1053	29.524	41.697	131.505	1	.000
[Group=3]Breastfeeding supplemented	-.720	1.1228	-2.920	1.481	.411	1	.522
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	-.154	.0253	-.203	-.104	36.896	1	.000
[Group=3] * Timeinweeks	.098	.0396	.021	.176	6.184	1	.013
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.167	.1187	-.066	.400	1.977	1	.160
CD4	.005	.0039	-.003	.012	1.556	1	.212
breastfeedingduration	-.447	1.4060	-3.202	2.309	.101	1	.751
(Scale)	18.977						

Dependent Variable: Lean body mass

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16q: Parameter Estimates for a GEE model for FM in breastfeeding mothers with low to normal BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	16.533	3.8162	9.054	24.013	18.770	1	.000
[Group=3] Breastfeeding supplemented	1.709	1.0764	-.400	3.819	2.522	1	.112
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.190	.0375	.116	.263	25.606	1	.000
[Group=3] * Timeinweeks	-.118	.0665	-.248	.012	3.147	1	.076
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	.036	.1224	-.204	.276	.088	1	.767
CD4	-.004	.0067	-.017	.009	.368	1	.544
breastfeedingduration	-.500	1.2436	-2.938	1.937	.162	1	.687
(Scale)	22.166						

Dependent Variable: Fat mass

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Table 16r: Parameter Estimates for a GEE model for percentage FM in breastfeeding mothers with low to normal BMI

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig
(Intercept)	30.988	4.9340	21.317	40.658	39.445	1	.000
[Group=3] Breastfeeding supplemented	2.310	1.5568	-.741	5.362	2.202	1	.138
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.298	.0460	.207	.388	41.885	1	.000
[Group=3] * Timeinweeks	-.191	.0827	-.353	-.029	5.319	1	.021
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
baselineage	-.034	.1693	-.366	.298	.041	1	.840
CD4	-.007	.0085	-.023	.010	.652	1	.419
breastfeedingduration	-.251	1.4376	-3.068	2.567	.030	1	.862
(Scale)	37.007						

Dependent Variable: Percentage Fat mass

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, baselineage, CD4, breastfeedingduration

a Set to zero because this parameter is redundant. Breastfeeding duration 1 (≤3.5 months) vs 0 (>3.5 months)

Appendix 17

Generalised estimating equations with exchangeable covariance matrix to assess longitudinal changes in laboratory parameters

Table 17d: Parameter Estimates for the mean change in CD4 count in the breastfeeding groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig
(Intercept)	500.999	85.1472	334.113	667.884	34.620	1	.000
[Group=3]	-10.957	35.8372	-81.197	59.282	.093	1	.760
[Group=2]	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-74.361	36.1297	-145.174	-3.548	4.236	1	.040
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
age	-.810	3.0567	-6.801	5.181	.070	1	.791
(Scale)	58807.330						

Dependent Variable: CD4
Model: (Intercept), Group, breastfeedingduration, age
a Set to zero because this parameter is redundant.

Appendix 18

GLM with Poisson distribution to compare incidence rates of opportunistic infections in the two groups of breastfeeding mothers

Table 19c: Parameter Estimates for a GLM for the event of oral thrush over follow-up period in both groups of breastfeeding mothers

Parameter	B(a)	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper				Wald Chi-Square	df	Sig.
(Intercept)	-39.807	1.0000	-41.767	-37.847	1584.571	1	.000	5.15E-018	7.26E-019	3.66E-017
[Group=3]Breastfeeding supplemented	.000	.	.	.	.	.	.	1.000	.000	.000
[Group=2]Breastfeeding control	0(b)	.	.	.	.	.	.	1	.	.
[durationbf=1.00]	1.861	1.4142	-.911	4.633	1.731	1	.188	6.429	.402	102.776
[durationbf=.00]	0(b)	.	.	.	.	.	.	1	.	.
(Scale)	1(c)									

Dependent Variable: ORALTHRUSH_sum
Model: (Intercept), Group, durationbf, offset = Followuptimeweeks
a Some parameter estimates are set to zero because the Hessian matrix is singular.
b Set to zero because this parameter is redundant.
c Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 19d: Parameter Estimates for a GLM for the event of PID over follow-up period in both groups of breastfeeding mothers

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-34.498	1.7022	-37.834	-31.161	410.750	1	.000	1.04E-015	3.71E-017	2.93E-014
[Group=3]Breastfeeding supplemented	-.555	.5430	-1.619	.509	1.044	1	.307	.574	.198	1.664
[Group=2]Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[durationbf=1.00]	1.749	.5578	.656	2.843	9.833	1	.002	5.750	1.927	17.161
[durationbf=.00]	0(a)	.	.	.	.	.	.	1	.	.
CD4	-.001	.0016	-.004	.002	.282	1	.595	.999	.996	1.002
Age	-.145	.0665	-.275	-.015	4.770	1	.029	.865	.759	.985
(Scale)	1(b)									

Dependent Variable: SignsofPID_sum
Model: (Intercept), Group, durationbf, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 19e: Parameter Estimates for a GLM for the event of LRTI over follow-up period in both groups of breastfeeding mothers

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.106	2.0487	-40.121	-32.090	310.600	1	.000	2.09E-016	3.76E-018	1.16E-014
[Group=3]Breastfeeding supplemented	.609	.7195	-.802	2.019	.715	1	.398	1.838	.449	7.528
[Group=2]Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[durationbf=1.00]	.785	.6673	-.523	2.093	1.383	1	.240	2.192	.593	8.107
[durationbf=.00]	0(a)	.	.	.	.	.	.	1	.	.
CD4	-.005	.0027	-.010	.001	2.730	1	.098	.995	.990	1.001
Age	-.046	.0705	-.184	.093	.420	1	.517	.955	.832	1.097
(Scale)	1(b)									

Dependent Variable: SignsofLRTI_sum
Model: (Intercept), Group, durationbf, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 19f: Parameter Estimates for a GLM for the event of hospital admission over follow-up period in both groups of breastfeeding mothers

Parameter	B(a)	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-29.309	6.6586	-42.360	-16.258	19.375	1	.000	1.87E-013	4.01E-019	8.69E-008
[Group=3] Breastfeeding supplemented	.000	.	.	.	.	.	.	1.000	.000	.000
[Group=2] Breastfeeding control	0(b)	.	.	.	.	.	.	1	.	.
[durationbf=1.00]	2.415	1.6201	-.761	5.590	2.221	1	.136	11.186	.467	267.747
[durationbf=.00]	0(b)	.	.	.	.	.	.	1	.	.
CD4	-.001	.0043	-.010	.007	.078	1	.780	.999	.990	1.007
Age	-.458	.3180	-1.082	.165	2.079	1	.149	.632	.339	1.179
(Scale)	1(c)									

Dependent Variable: HOAdmissions_sum
Model: (Intercept), Group, durationbf, CD4, age, offset = Followuptimeweeks
a Some parameter estimates are set to zero because the Hessian matrix is singular.
b Set to zero because this parameter is redundant.
c Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 19g: Parameter Estimates for a GLM for the event of breast pathology over follow-up period in both groups of breastfeeding mothers

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-35.542	1.7656	-39.003	-32.082	405.227	1	.000	3.67E-016	1.15E-017	1.17E-014
[Group=3] Breastfeeding supplemented	.096	.5824	-1.046	1.237	.027	1	.870	1.100	.351	3.446
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[durationbf=1.00]	2.116	.6244	.892	3.339	11.478	1	.001	8.294	2.439	28.203
[durationbf=.00]	0(a)	.	.	.	.	.	.	1	.	.
CD4	.000	.0017	-.003	.003	.004	1	.948	1.000	.997	1.003
Age	-.142	.0687	-.277	-.008	4.296	1	.038	.867	.758	.992
(Scale)	1(b)									

Dependent Variable: Breast_sum
Model: (Intercept), Group, durationbf, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 19h: Parameter Estimates for a GLM for the event of genital ulcers over follow-up period in both groups of breastfeeding mothers

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-35.185	.7681	-36.690	-33.679	2098.317	1	.000	5.24E-016	1.16E-016	2.36E-015
[Group=3]Breastfeeding supplemented	-.561	.2464	-1.044	-.078	5.174	1	.023	.571	.352	.925
[Group=2]Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[durationbf=1.00]	.976	.2519	.482	1.469	14.994	1	.000	2.653	1.619	4.346
[durationbf=.00]	0(a)	.	.	.	.	.	.	1	.	.
CD4	.001	.0006	-9.89E-005	.002	3.174	1	.075	1.001	1.000	1.002
Age	-.067	.0287	-.124	-.011	5.515	1	.019	.935	.884	.989
(Scale)	1(b)									

Dependent Variable: genital_sum
Model: (Intercept), Group, durationbf, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 19i: Parameter Estimates for a GLM for the event of herpes simplex over follow-up period in both groups of breastfeeding mothers

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-40.079	3.6004	-47.136	-33.023	123.921	1	.000	3.92E-018	3.38E-021	4.55E-015
[Group=3]Breastfeeding supplemented	.554	1.1970	-1.792	2.900	.214	1	.643	1.741	.167	18.183
[Group=2]Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[durationbf=1.00]	2.388	1.2552	-.072	4.848	3.620	1	.057	10.892	.930	127.509
[durationbf=.00]	0(a)	.	.	.	.	.	.	1	.	.
CD4	.002	.0024	-.003	.007	.779	1	.377	1.002	.997	1.007
Age	-.070	.1324	-.329	.190	.277	1	.598	.933	.719	1.209
(Scale)	1(b)									

Dependent Variable: Herpessimplex_sum
Model: (Intercept), Group, durationbf, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 19j: Parameter Estimates for a GLM for the event of SRQ20 score≥ 8 over follow-up period in both groups of breastfeeding mothers

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.257	1.3650	-38.932	-33.581	705.563	1	.000	1.79E-016	1.24E-017	2.60E-015
[Group=3] Breastfeeding supplemented	.884	.4904	-.077	1.845	3.249	1	.071	2.421	.926	6.330
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[durationbf=1.00]	.736	.4449	-.136	1.608	2.734	1	.098	2.087	.873	4.991
[durationbf=.00]	0(a)	.	.	.	.	.	.	1	.	.
CD4	.000	.0011	-.002	.002	.050	1	.823	1.000	.998	1.002
Age	-.088	.0516	-.189	.013	2.903	1	.088	.916	.828	1.013
(Scale)	1(b)									

Dependent Variable: depsrq_sum
Model: (Intercept), Group, durationbf, CD4, age, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Appendix 19

GEE models for anthropometric measures in the infants of the breastfeeding infants

Table 20e: Parameter Estimates for a GEE model for weight change over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	3.221	.4013	2.434	4.007	64.415	1	.000
[Group=3]Breastfeeding supplemented	-.050	.1223	-.290	.190	.168	1	.682
[Group=2]Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.164	.0052	.154	.174	1002.872	1	.000
[Group=3] * Timeinweeks	-.011	.0068	-.025	.002	2.715	1	.099
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.430	.1531	-.730	-.130	7.890	1	.005
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
MomCD4	-3.85E-006	.0002	.000	.000	.000	1	.985
MomBMI	.033	.0134	.007	.059	6.029	1	.014
(Scale)	.897						

Dependent Variable: weight2
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI
a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20f: Parameter Estimates for a GEE model for length over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	49.446	1.3444	46.811	52.081	1352.713	1	.000
[Group=3] Breastfeeding supplemented	.257	.3971	-.522	1.035	.418	1	.518
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.571	.0100	.552	.591	3290.229	1	.000
[Group=3] * Timeinweeks	-.011	.0133	-.037	.015	.694	1	.405
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.921	.4579	-1.818	-.023	4.043	1	.044
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
MomCD4	.001	.0005	.000	.002	2.943	1	.086
MomBMI	.062	.0426	-.022	.145	2.107	1	.147
(Scale)	7.140						

Dependent Variable: length
Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI
a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20g: Parameter Estimates for a GEE model for head circumference over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	35.616	.7745	34.097	37.134	2114.371	1	.000
[Group=3] Breastfeeding supplemented	.246	.2399	-.225	.716	1.048	1	.306
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.254	.0065	.241	.266	1506.201	1	.000
[Group=3] * Timeinweeks	.005	.0078	-.011	.020	.350	1	.554
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.466	.2412	-.939	.007	3.736	1	.053
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
MomCD4	.000	.0003	-.001	.001	.168	1	.682
MomBMI	.045	.0258	-.005	.096	3.098	1	.078
(Scale)	2.458						

Dependent Variable: hc

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20h: Parameter Estimates for a GEE model for MUAC over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	10.895	.4777	9.959	11.831	520.157	1	.000
[Group=3] Breastfeeding supplemented	-.203	.1776	-.551	.145	1.305	1	.253
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.125	.0059	.114	.137	455.204	1	.000
[Group=3] * Timeinweeks	-.007	.0081	-.023	.009	.677	1	.411
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.533	.1905	-.906	-.159	7.814	1	.005
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
MomCD4	.000	.0003	-.001	.000	.860	1	.354
MomBMI	.030	.0158	-.001	.061	3.557	1	.059
(Scale)	1.515						

Dependent Variable: ac

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20i: Parameter Estimates for a GEE model for TSF over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	8.785	.7022	7.409	10.162	156.515	1	.000
[Group=3] Breastfeeding supplemented	-.354	.2540	-.852	.144	1.941	1	.164
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.107	.0097	.088	.126	121.532	1	.000
[Group=3] * Timeinweeks	-.019	.0133	-.045	.007	2.068	1	.150
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.853	.2770	-1.396	-.310	9.482	1	.002
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
MomCD4	.000	.0004	-.001	.000	.994	1	.319
MomBMI	-.005	.0242	-.053	.042	.045	1	.831
(Scale)	3.853						

Dependent Variable: ts

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20j: Parameter Estimates for a GEE model for BMI over time in breastfeeding infants of both groups

	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
Parameter	Lower	Upper	Wald Chi-Square	df	Sig.	Lower	Upper
(Intercept)	14.918	.6871	13.571	16.265	471.437	1	.000
[Group=3] Breastfeeding supplemented	-.384	.2710	-.916	.147	2.012	1	.156
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.119	.0094	.101	.138	161.185	1	.000
[Group=3] * Timeinweeks	-.014	.0124	-.038	.011	1.201	1	.273
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.685	.2840	-1.242	-.128	5.819	1	.016
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
MomCD4	.000	.0004	-.001	.000	1.254	1	.263
MomBMI	.043	.0243	-.005	.091	3.102	1	.078
(Scale)	3.585						

Dependent Variable: BMI

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20k: Parameter Estimates for a GEE model for weight/age z score over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-.625	.5028	-1.610	.361	1.545	1	.214
[Group=3] Breastfeeding supplemented	-.067	.1786	-.418	.283	.143	1	.706
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.022	.0057	.010	.033	14.349	1	.000
[Group=3] * Timeinweeks	-.008	.0075	-.023	.006	1.292	1	.256
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduratio n=1.00]	-.688	.1898	-1.060	-.316	13.135	1	.000
[breastfeedingduratio n=.00]	0(a)	.	.	.	.	.	.
MomCD4	-1.45E-005	.0002	.000	.000	.005	1	.946
MomBMI	.028	.0163	-.004	.060	2.886	1	.089
(Scale)	1.034						

Dependent Variable: zwaz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20l: Parameter Estimates for a GEE model for length/age z score over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-1.208	.5823	-2.349	-.067	4.303	1	.038
[Group=3] Breastfeeding supplemented	.172	.1862	-.193	.537	.854	1	.356
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.011	.0040	.003	.019	7.793	1	.005
[Group=3] * Timeinweeks	-.003	.0056	-.015	.008	.380	1	.538
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration= 1.00]	-.580	.2122	-.996	-.164	7.462	1	.006
[breastfeedingduration= .00]	0(a)	.	.	.	.	.	.
MomCD4	.000	.0002	-8.62E-005	.001	2.391	1	.122
MomBMI	.016	.0184	-.020	.052	.800	1	.371
(Scale)	1.108						

Dependent Variable: zhaz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20m: Parameter Estimates for a GEE model for BMI/age z score over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	.096	.4478	-.782	.973	.046	1	.831
[Group=3] Breastfeeding supplemented	-.235	.1812	-.590	.120	1.683	1	.194
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.020	.0060	.008	.032	10.974	1	.001
[Group=3] * Timeinweeks	-.008	.0080	-.024	.008	.992	1	.319
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.489	.1827	-.847	-.131	7.159	1	.007
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
MomCD4	.000	.0002	-.001	.000	1.131	1	.287
MomBMI	.025	.0159	-.006	.056	2.434	1	.119
(Scale)	1.184						

Dependent Variable: zBMI

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20n: Parameter Estimates for a GEE model for weight/height z score over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	.577	.4375	-.280	1.435	1.741	1	.187
[Group=3] Breastfeeding supplemented	-.295	.1880	-.664	.073	2.470	1	.116
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.013	.0056	.002	.024	5.091	1	.024
[Group=3] * Timeinweeks	-.006	.0079	-.022	.009	.586	1	.444
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.333	.1846	-.695	.029	3.253	1	.071
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
MomCD4	.000	.0002	-.001	.000	1.975	1	.160
MomBMI	.018	.0160	-.013	.049	1.290	1	.256
(Scale)	1.245						

Dependent Variable: zwhz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20o: Parameter Estimates for a GEE model for head circumference/age z score over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	2.827	3.1178	-3.284	8.937	.822	1	.365
[Group=3] Breastfeeding supplemented	4.786	4.5315	-4.096	13.667	1.115	1	.291
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.405	.3984	-.376	1.186	1.033	1	.309
[Group=3] * Timeinweeks	-.403	.3996	-1.186	.381	1.015	1	.314
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-1.590	1.0837	-3.714	.534	2.152	1	.142
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
MomCD4	.000	.0009	-.001	.002	.213	1	.645
MomBMI	-.246	.2626	-.761	.269	.878	1	.349
(Scale)	1101.950						

Dependent Variable: zhcz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20p: Parameter Estimates for a GEE model for MUAC/age z score over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-.277	.5133	-1.283	.729	.290	1	.590
[Group=3] Breastfeeding supplemented	-.431	.2716	-.963	.102	2.514	1	.113
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.010	.0053	.000	.021	3.758	1	.053
[Group=3] * Timeinweeks	.006	.0092	-.012	.024	.439	1	.508
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.655	.2072	-1.061	-.249	9.990	1	.002
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
MomCD4	.000	.0002	-.001	.000	1.634	1	.201
MomBMI	.030	.0170	-.003	.063	3.158	1	.076
(Scale)	1.039						

Dependent Variable: zacz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 20q: Parameter Estimates for a GEE model for TSF/age z score over time in breastfeeding infants of both groups

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	.461	.5002	-.520	1.441	.849	1	.357
[Group=3] Breastfeeding supplemented	-.581	.2528	-1.076	-.085	5.277	1	.022
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.020	.0050	.010	.029	15.037	1	.000
[Group=3] * Timeinweeks	.005	.0090	-.013	.022	.285	1	.593
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.530	.1996	-.922	-.139	7.056	1	.008
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
MomCD4	.000	.0003	-.001	.000	.500	1	.479
MomBMI	.005	.0175	-.030	.039	.069	1	.793
(Scale)	.945						

Dependent Variable: ztsz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration, MomCD4, MomBMI

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Appendix 20

GLM models using Poisson distribution to compare incidence rates of opportunistic infections between the two breastfeeding groups of infants

Table 21e: Parameter Estimates for a GLM for the event of oral thrush amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
	Lower	Upper			Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-37.109	.2279	-37.555	-36.662	26516.681	1	.000	7.65E-017	4.90E-017	1.20E-016
[Group=3] Breastfeeding supplemented	.223	.2941	-.353	.800	.576	1	.448	1.250	.702	2.225
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration =1.00]	.416	.3065	-.185	1.016	1.838	1	.175	1.515	.831	2.763
[breastfeedingduration =.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: ORALTHRUSH_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 21f: Parameter Estimates for a GLM for the event of diarrhoea amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.584	.1789	-36.935	-36.234	41819.334	1	.000	1.29E-016	9.11E-017	1.84E-016
[Group=3] Breastfeeding supplemented	.267	.2353	-.194	.728	1.287	1	.257	1.306	.823	2.072
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	.021	.2656	-.499	.542	.006	1	.936	1.021	.607	1.719
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: HOdiarrhoea_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 21g: Parameter Estimates for a GLM for the event of ear discharge amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-38.411	.4613	-39.316	-37.507	6932.606	1	.000	2.08E-017	8.42E-018	5.14E-017
[Group=3] Breastfeeding supplemented	-.862	.8505	-2.528	.805	1.026	1	.311	.423	.080	2.237
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	-.448	1.0979	-2.600	1.704	.166	1	.683	.639	.074	5.496
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: HOeardischarge_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 21h: Parameter Estimates for a GLM for the event of LRTI amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
	Lower	Upper			Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-39.188	.5898	-40.344	-38.033	4415.430	1	.000	9.56E-018	3.01E-018	3.04E-017
[Group=3] Breastfeeding supplemented	.161	.6454	-1.104	1.426	.063	1	.803	1.175	.332	4.164
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	1.653	.6454	.388	2.918	6.560	1	.010	5.223	1.474	18.505
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: SignsofLRTI_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 21i: Parameter Estimates for a GLM for the event of PTB amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	. Wald Chi-Square	df	Sig		Lower	Upper
(Intercept)	-38.218	.4205	-39.042	-37.394	8260.820	1	.000	2.52E-017	1.11E-017	5.76E-017
[Group=3] Breastfeeding supplemented	-2.056	1.0845	-4.182	.070	3.594	1	.058	.128	.015	1.072
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	.463	.8283	-1.160	2.087	.313	1	.576	1.589	.313	8.059
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: tb_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 21j: Parameter Estimates for a GLM for the event of FTT amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-39.523	.7295	-40.952	-38.093	2935.427	1	.000	6.85E-018	1.64E-018	2.86E-017
[Group=3] Breastfeeding supplemented	1.093	.8177	-.509	2.696	1.788	1	.181	2.985	.601	14.822
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	.679	.6841	-.661	2.020	.986	1	.321	1.973	.516	7.540
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: ftt_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 21k: Parameter Estimates for a GLM for the event of skin sepsis amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-38.079	.3574	-38.780	-37.379	11353.523	1	.000	2.90E-017	1.44E-017	5.84E-017
[Group=3] Breastfeeding supplemented	.300	.4341	-.551	1.151	.478	1	.489	1.350	.577	3.161
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	.894	.4217	.067	1.720	4.492	1	.034	2.444	1.070	5.587
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: skinsepsis_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 21l: Parameter Estimates for a GLM for the event of meningitis amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-40.382	1.1402	-42.617	-38.147	1254.389	1	.000	2.90E-018	3.10E-019	2.71E-017
[Group=3] Breastfeeding supplemented	-.288	1.4606	-3.150	2.575	.039	1	.844	.750	.043	13.132
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	1.204	1.4606	-1.659	4.067	.679	1	.410	3.333	.190	58.363
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: meningitis_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 21m: Parameter Estimates for a GLM for the event of developmental delay amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-39.022	.5171	-40.036	-38.009	5694.953	1	.000	1.13E-017	4.10E-018	3.11E-017
[Group=3] Breastfeeding supplemented	.275	.5059	-.716	1.267	.296	1	.586	1.317	.489	3.550
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	2.096	.5341	1.049	3.143	15.405	1	.000	8.135	2.856	23.173
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: development_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 21n: Parameter Estimates for a GLM for the event of hospital admission amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-39.657	.6996	-41.028	-38.286	3212.969	1	.000	5.99E-018	1.52E-018	2.36E-017
[Group=3] Breastfeeding supplemented	.504	.6934	-.855	1.863	.529	1	.467	1.656	.425	6.446
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	1.996	.6934	.637	3.355	8.285	1	.004	7.359	1.891	28.649
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: HOAdmissions_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 21o: Parameter Estimates for a GLM for the event of death amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-40.291	1.0509	-42.351	-38.231	1470.066	1	.000	3.18E-018	4.05E-019	2.49E-017
[Group=3] Breastfeeding supplemented	-.551	1.0235	-2.557	1.455	.290	1	.590	.576	.078	4.283
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	2.368	1.1818	.052	4.684	4.015	1	.045	10.677	1.053	108.252
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: Child status

Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks

a Set to zero because this parameter is redundant.

b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Appendix 21

GEE Models using exchangeable covariance matrix for growth in the breastfed HIV positive infants

Table 22b: Parameter Estimates for a GEE model for weight change over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	3.971	.3529	3.279	4.663	126.618	1	.000
[Group=3] Breastfeeding supplemented	-.298	.3121	-.910	.314	.913	1	.339
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.122	.0220	.079	.165	30.711	1	.000
[Group=3] * Timeinweeks	-.004	.0242	-.051	.044	.022	1	.881
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.340	.3175	-.962	.282	1.148	1	.284
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	.741						

Dependent Variable: weight2

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22c: Parameter Estimates for a GEE model for length change over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig
(Intercept)	50.455	.9553	48.583	52.328	2789.250	1	.000
[Group=3] Breastfeeding supplemented	-.561	1.0488	-2.616	1.495	.286	1	.593
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.500	.0363	.429	.571	189.243	1	.000
[Group=3] * Timeinweeks	-.006	.0430	-.091	.078	.021	1	.884
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	.138	.9384	-1.701	1.977	.022	1	.883
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	4.978						

Dependent Variable: length

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22d: Parameter Estimates a GEE model for head circumference change over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig
(Intercept)	36.577	.5601	35.479	37.674	4263.833	1	.000
[Group=3] Breastfeeding supplemented	-.901	.5019	-1.885	.082	3.227	1	.072
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.234	.0248	.185	.283	88.856	1	.000
[Group=3] * Timeinweeks	-.002	.0308	-.063	.058	.005	1	.943
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	.049	.5460	-1.022	1.119	.008	1	.929
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	2.072						

Dependent Variable: hc

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22e: Parameter Estimates for a GEE model for MUAC change over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig
(Intercept)	11.785	.4963	10.812	12.757	563.892	1	.000
[Group=3] Breastfeeding supplemented	-.457	.5731	-1.581	.666	.637	1	.425
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.073	.0270	.020	.126	7.261	1	.007
[Group=3] * Timeinweeks	.024	.0311	-.037	.085	.576	1	.448
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.919	.4192	-1.740	-.097	4.801	1	.028
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	1.473						

Dependent Variable: ac

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22f: Parameter Estimates for a GEE model for TSF over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig
(Intercept)	9.521	.5565	8.430	10.612	292.683	1	.000
[Group=3] Breastfeeding supplemented	-.631	.6538	-1.913	.650	.932	1	.334
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.044	.0242	-.004	.091	3.292	1	.070
[Group=3] * Timeinweeks	.011	.0317	-.051	.073	.129	1	.719
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-2.030	.4294	-2.872	-1.188	22.346	1	.000
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	2.622						

Dependent Variable: ts

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22g: Parameter Estimates for a GEE model for length/age z score over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-.807	.4905	-1.768	.155	2.704	1	.100
[Group=3] Breastfeeding supplemented	-.084	.6163	-1.292	1.124	.019	1	.891
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	-.013	.0134	-.040	.013	.983	1	.321
[Group=3] * Timeinweeks	-.016	.0189	-.053	.021	.745	1	.388
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.445	.5000	-1.425	.535	.791	1	.374
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	1.035						

Dependent Variable: zhaz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22h: Parameter Estimates for a GEE model for weight/age z score over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	.168	.5950	-.998	1.334	.080	1	.777
[Group=3] Breastfeeding supplemented	-.420	.5630	-1.524	.683	.557	1	.455
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	-.023	.0200	-.062	.017	1.261	1	.261
[Group=3] * Timeinweeks	-.003	.0251	-.053	.046	.019	1	.891
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.916	.4773	-1.852	.019	3.687	1	.055
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	1.330						

Dependent Variable: zwaz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22i: Parameter Estimates for a GEE model for BMI change over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	15.804	.7387	14.356	17.252	457.717	1	.000
[Group=3] Breastfeeding supplemented	-.848	.5469	-1.920	.224	2.404	1	.121
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.071	.0246	.023	.119	8.305	1	.004
[Group=3] * Timeinweeks	.010	.0292	-.047	.068	.128	1	.721
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-1.072	.5399	-2.130	-.014	3.944	1	.047
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	2.441						

Dependent Variable: BMI

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22j: Parameter Estimates for a GEE model for BMI/age z score over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	.817	.4819	-.128	1.761	2.872	1	.090
[Group=3] Breastfeeding supplemented	-.545	.3824	-1.294	.205	2.031	1	.154
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	-.017	.0168	-.050	.016	1.027	1	.311
[Group=3] * Timeinweeks	.009	.0205	-.032	.049	.178	1	.673
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.912	.3588	-1.615	-.209	6.463	1	.011
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	1.072						

Dependent Variable: zBMI

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22k: Parameter Estimates for a GEE model for weight/length z score over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	1.281	.3161	.662	1.901	16.426	1	.000
[Group=3] Breastfeeding supplemented	-.456	.3196	-1.083	.170	2.037	1	.154
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	-.029	.0181	-.064	.007	2.548	1	.110
[Group=3] * Timeinweeks	.009	.0212	-.033	.050	.173	1	.678
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.817	.2973	-1.400	-.234	7.553	1	.006
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	.928						

Dependent Variable: zwhz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22l: Parameter Estimates for a GEE model for head circumference/age z score over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	.607	.4440	-.263	1.477	1.870	1	.171
[Group=3] Breastfeeding supplemented	-.537	.4636	-1.446	.372	1.341	1	.247
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	-.013	.0179	-.048	.022	.505	1	.477
[Group=3] * Timeinweeks	-.011	.0244	-.059	.037	.199	1	.655
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-.513	.4212	-1.338	.313	1.481	1	.224
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	1.201						

Dependent Variable: zhcz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22m: Parameter Estimates for a GEE model for MUAC/age z score over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	.540	.6704	-.774	1.853	.648	1	.421
[Group=3] Breastfeeding supplemented	-2.035	1.3982	-4.776	.705	2.119	1	.145
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	-.032	.0310	-.093	.029	1.076	1	.300
[Group=3] * Timeinweeks	.094	.0551	-.014	.202	2.924	1	.087
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-1.226	.4715	-2.150	-.302	6.765	1	.009
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	1.708						

Dependent Variable: zacz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 22n: Parameter Estimates for a GEE model for TSF/age z score over time in the infected infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	.788	.3550	.092	1.483	4.921	1	.027
[Group=3] Breastfeeding supplemented	-1.762	1.3087	-4.327	.803	1.812	1	.178
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.
Timeinweeks	.000	.0145	-.028	.029	.000	1	.988
[Group=3] * Timeinweeks	.062	.0501	-.036	.160	1.535	1	.215
[Group=2] * Timeinweeks	0(a)	.	.	.	.	.	.
[breastfeedingduration=1.00]	-1.522	.3171	-2.143	-.900	23.027	1	.000
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.
(Scale)	1.036						

Dependent Variable: ztsz

Model: (Intercept), Group, Timeinweeks, Group * Timeinweeks, breastfeedingduration

a Set to zero because this parameter is redundant. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Appendix 22

GLM using Poisson distribution to calculate incidence rates for opportunistic infections in the HIV infected breastfed infants

Table 23d: Parameter Estimates for a GLM for the event of oral thrush amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.177	.5497	-37.254	-35.100	4331.469	1	.000	1.94E-016	6.62E-017	5.71E-016
[Group=3] Breastfeeding supplemented	-.120	.6146	-1.324	1.085	.038	1	.845	.887	.266	2.958
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	.395	.6427	-.865	1.654	.377	1	.539	1.484	.421	5.229
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: ORALTHRUSH_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 23e: Parameter Estimates for a GLM for the event of ear discharge amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.671	.7680	-38.177	-35.166	2280.058			1.19E-016	2.63E-017	5.34E-016
[Group=3] Breastfeeding supplemented	-.784	1.2838	-3.300	1.733	.373	1	.542	.457	.037	5.655
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	-.784	1.2838	-3.300	1.733	.373	1	.542	.457	.037	5.655
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: HOeardischarge_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 23f: Parameter Estimates for a GLM for the event of diarrhoea amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.221	.5967	-37.391	-35.051	3684.133	1	.000	1.86E-016	5.77E-017	5.99E-016
[Group=3] Breastfeeding supplemented	-.992	.7614	-2.484	.500	1.698	1	.193	.371	.083	1.649
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	.481	.7614	-1.011	1.974	.399	1	.527	1.618	.364	7.196
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: HOdiarrhoea_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 23g: Parameter Estimates for a GLM for the event of LRTI amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.897	.7663	-38.399	-35.395	2318.048	1	.000	9.46E-017	2.11E-017	4.25E-016
[Group=3] Breastfeeding supplemented	-.050	.7613	-1.542	1.442	.004	1	.948	.951	.214	4.230
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	.778	.8512	-.890	2.446	.835	1	.361	2.177	.410	11.544
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: SignsofLRTI_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 23h: Parameter Estimates for a GLM for the event of FTT amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-36.916	.7071	-38.302	-35.530	2725.625	1	.000	9.28E-017	2.32E-017	3.71E-016
[Group=3] Breastfeeding supplemented	-.336	1.0000	-2.296	1.623	.113	1	.737	.714	.101	5.071
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: ftt_sum
Model: (Intercept), Group, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 23i: Parameter Estimates for a GLM for the event of PTB amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-35.819	.5130	-36.825	-34.814	4875.841	1	.000	2.78E-016	1.02E-016	7.60E-016
[Group=3] Breastfeeding supplemented	-1.777	1.1307	-3.993	.439	2.469	1	.116	.169	.018	1.552
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	-.534	.8939	-2.286	1.218	.356	1	.551	.587	.102	3.382
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: tb_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 23j: Parameter Estimates for a GLM for the event of developmental delay amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-38.189	1.1230	-40.390	-35.989	1156.533	1	.000	2.60E-017	2.88E-018	2.35E-016
[Group=3] Breastfeeding supplemented	1.087	.7694	-.421	2.595	1.996	1	.158	2.965	.656	13.395
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	2.006	1.0485	-.049	4.061	3.659	1	.056	7.430	.952	58.008
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: development_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 23k: Parameter Estimates for a GLM for the event of hospital admission amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-37.034	.8244	-38.650	-35.419	2018.157	1	.000	8.24E-017	1.64E-017	4.15E-016
[Group=3] Breastfeeding supplemented	.273	.9078	-1.506	2.052	.090	1	.764	1.314	.222	7.783
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	.273	.9078	-1.506	2.052	.090	1	.764	1.314	.222	7.783
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: HOAdmissions_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 23l: Parameter Estimates for a GLM for the event of death amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
(Intercept)	-37.410	1.0378	-39.443	-35.376	1299.46	1	.000	5.67E-017	7.41E-018	4.33E-016
[Group=3] Breastfeeding supplemented	-.603	1.0382	-2.638	1.431	.338	1	.561	.547	.071	4.185
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	.953	1.1988	-1.396	3.303	.632	1	.427	2.594	.247	27.189
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: Child status
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months

Table 23m: Parameter Estimates for a GLM for the event of seborrhaeic dermatitis amongst the breastfed infants

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-Square.	df	Sig		Lower	Upper
(Intercept)	-35.790	.5069	-36.784	-34.797	4985.850	1	.000	2.86E-016	1.06E-016	7.73E-016
[Group=3] Breastfeeding supplemented	-2.106	1.1082	-4.278	.066	3.612	1	.057	.122	.014	1.068
[Group=2] Breastfeeding control	0(a)	.	.	.	.	.	.	1	.	.
[breastfeedingduration=1.00]	-.070	.7836	-1.606	1.466	.008	1	.929	.933	.201	4.332
[breastfeedingduration=.00]	0(a)	.	.	.	.	.	.	1	.	.
(Scale)	1(b)									

Dependent Variable: sebderm_sum
Model: (Intercept), Group, breastfeedingduration, offset = Followuptimeweeks
a Set to zero because this parameter is redundant.
b Fixed at the displayed value. Duration of breastfeeding 0:>3.5 Months; 1:≤ 3.5 months