EFFECT OF MATERNAL HIV STATUS ON BREAST MILK INTAKE AND GROWTH OF HIV-UNINFECTED KENYAN INFANTS AT 6 WEEKS POST-PARTUM AND 6 MONTHS OF AGE

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DEDICATION

To my wife Barbara Andisi Oiye. To my sons, Myles Okinyi Oiye & Rowell Sande Oiye. Also to my unborn children, grandchildren and great grandchildren.

To my parents Engineer Gilbert Oiye and Brigit Oiye, for their obsession with further education.

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ACRONYMS AND ABBREVIATIONS

AED	Academy for Education and Development
AFASS	Acceptable Feasible Affordable Sustainable and Safe
AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal clinic
ART	Antiretroviral Therapy
ARV	Antiretroviral
AZT	Azidothymidine
BMI	Body Mass Index
CD4	Cluster of differentiation 4
CDC	Centre for Disease Control
CTX	Cotrimoxazole
EBF	Exclusive breastfeeding
ELISA	Enzyme-linked immune sorbent assay
EU	Exposed, uninfected
FTIR	Fourier transform infrared spectrometry
GOK	Government of Kenya
HAART	Highly Active Antiretroviral Therapy
HFIAS	Household Food Insecurity and Access Scale
HIV	Human Immuno deficiency Virus
HIV-EU	HIV- exposed, uninfected
HIV-U	HIV-uninfected
IAEA	International Atomic Energy Agency
ICAP	International Centre for Aids Care and Control
IOM	Institute of Medicine
IRMS	Isotope ratio mass spectrometry
KDHS	Kenya Demographic Health Survey
KEMRI	Kenya Medical Research Institute
MCH	Maternal and Child Health
MUAC	Mid-upper Arm Circumference
NVP	Nevirapine

OIs	Opportunistic Infections
PCP	*pneumocystis jiroveccii* pneumonia prophylaxis
PCR	Polymerase chain reaction
PICT	Provider initiated counselling and testing
PMTCT	Prevention of Mother-to-Child Transmission
SD	Standard Deviation
UNAIDS	United Nations Programme on HIV/AIDS
VCT	Voluntary counselling and testing
WHO	World Health Organisation
UNICEF	United Nations Children's Fund

DEFINITION OF OPERATIONAL TERMS

Breast milk	Liquid produced by human mammalian glands for the nourishment of the newborns
Breastfeeding	The act of providing breast milk to infants and young children
Exclusive breastfeeding	Provision of breast milk alone and not providing any other solid or liquid. Exclusive breastfeeding allows for provision of ORS, drops, syrups (vitamins, minerals, medicines).
Breast milk intake	The amount of breast milk taken by a breastfeeding infant and young child. This is measured in volume or weight per day
HIV-exposed uninfected infants	HIV-negative Infants born of HIV-positive mothers
HIV-unexposed infants	HIV-negative infants born of HIV-negative mothers
Growth of infants	Increase in height or weight of infants with age and as indicated by standard normal deviates (Z scores) using WHO Child Growth Standards

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ABSTRACT

Background: All infants irrespective of their HIV status and that of their mothers should be exclusively breastfed in their first half of infancy. It is however not well known if infants' exposure to maternal HIV infection does affect their breast milk intake, linear growth and body composition.

Objectives: To compare breast milk intake of HIV-uninfected infants born of HIV-positive mothers with that of infants born of HIV-negative mothers at 6 weeks and 6 months of age. Additionally, the study aimed to assess the effect of maternal HIV status on infant growth, body composition (lean mass) and breastfeeding practices among the mothers.

Methods: A prospective cohort study with cross sectional data collection at 6 weeks and 6 months post-partum. The study was based at the Maternal and Child Health Clinic of Siaya County Referral Hospital in the Lake Region of Western part of Kenya. Seventy five (75) HIV-1 positive and 68 HIV-1 negative mothers with HIV-uninfected infants were systematically sampled and recruited with their infants at 6 weeks post-partum and followed up at 6 months after birth. At recruitment and follow-up, mothers and their infants were tested of HIV. Excluded mother-infants dyads were those with preterm infants, infants <2500g, infants not able to breastfeed and mother or infants who were severely ill. Breast milk intake was measured using the deuterium dose-to-mother technique (isotopic technique) in which pre-dose (baseline) saliva samples were obtained from both the infant and the mother on day 0. Subsequently, baseline saliva collection, a 30g dose of deuterium oxide was given to the mothers. Post-dose saliva samples were collected from both infant and mother over 14 days on days 1, 2, 3, 4, 13 and 14. Infant length, weight and skin fold thickness (growth) were measured. Fat mass and fat free mass (body composition) was measured using deuterium-dose-to-the-infant technique in which a pre-dose saliva sample was collected from the infant after which a deuterium oxide dose (0.5g/kg body weight) was administered and post-dose saliva samples collected at 3 and 4 hours post-dose. To measure both infant breast milk intake and lean mass, deuterium oxide enrichment in saliva was measured using Fourier Transform Infrared (FTIR) Spectrophotometer. Total body water from the FTIR measurement was converted to breast milk water

volume and lean mass using standard equations and assumptions. Other data were collected using a standard questionnaire and included socio-economic data, demographic characteristics, infant feeding practices and care practices and infant and maternal anthropometries.

Results: There were no significant differences in breast milk intake between the two groups at 6 weeks and 6 months. At 6 weeks postpartum infants born of HIV positive mothers (HIV-EU) consumed 717g/day of breast milk and this was comparable to 712.6g/day consumed by infants born of HIV-negative mothers HIV-U (p=0.86). At 6 months after birth HIV-EU consumed 960.8g/day of breast milk while HIV-U consumed 963.1g/day and the two intakes were comparable (p=0.95). Factors positively associated with breast milk intake among HIV-uninfected infants were maternal BMI (r=0.247 at 6 weeks), maternal lean mass (r=0.270 at 6 weeks and r=0.365 at 6 months), infant birth weight (r=0.345 at 6 weeks) and infant current weight (r=0.486 at 6 weeks and r=0.557 at 6 months). At 6 weeks postpartum, the deuterium oxide determined exclusive breastfeeding was comparable between HIV-positive mothers (23.3%) and HIV-negative mothers (14.5%), p=0.21. At 6 months after birth the deuterium oxide EBF rates were significantly different (p=0.025) between the HIV-positive (43.3%) and HIV-negative mothers (24.2%). The self-recalled EBF rates were 4 times and 5 times higher than isotopic determine figures for HIV-EU and HIV-U respectively at 6 weeks postpartum. At 6 months after birth, the factors reduced to 1.7 and 2.5 times for HIV-EU and HIV-U respectively. At 6 weeks post-partum, there were significant differences between HIV-exposed uninfected and HIV-unexposed infants in length-for-age Z scores (1.0 for HIV-EU and 0.6 for HIV-E, p=0.011). At 6 months of age, there were no differences in mean LAZ (-1.2 for HIV-EU and -0.9 for HIV-U, p=0.154). There was no significant difference in infants lean mass and fat mass both at 6 weeks and 6 months of age. HIV-EU and the HIV-U had comparable fat free mass (5.7kg for HIV-EU and 5.9 kg for HIV-U, p=0.10), fat mass (1.6kg for HIV-EU and HIV-U, p=1.0), % fat mass (22.3% for HIV-EU and 21.3 for HIV-U, p=0.34), fat free mass index (14.7 kgm^{-2} for HIV-EU and 14.5 kgm^{-2} for HIV-U, p=0.73) and fat mass index (4.3 kgm^{-2} for HIV-EU and 4.0 kgm^{-2} for HIV-U, p=0.35). Among infants born of HIV-positive mothers, those

whose mothers were on ART had lower free fat mass (5.4kg verses 6.0kg for non-ART, p=0.018), and conversely higher % fat mass (24.0% versus 19.3% for non-ART, p=0.04) and lower free fat mass index (14.2kg verses 16.0kg for non-ART, p=0.076).

Conclusions: Within the exclusive breastfeeding age bracket in resource poor settings, maternal HIV status does not influence the breast milk intake of HIV-uninfected infants. Infants of HIV-positive mothers are however more likely to be exclusively breastfed compared to infants of HIV-negative mothers. Maternal recalls tend to over-estimate exclusive breastfeeding rate when compared to deuterium oxide dilution technique. Interventions that ensure normal maternal body mass index, maternal lean mass, infant size (birth and current weight) are important in increasing breast milk intake which is in turn important in promoting growth and lean mass of HIV-uninfected infants.

Recommendations: Validation of self-reported EBF practices with the low-cost, non-invasive deuterium oxide dilution technique is highly recommended to facilitate more effective breastfeeding promotion campaigns. Intensify breastfeeding messages among the HIV-negative mothers. They have been shown to exclusively breastfeed less as compared to the HIV positive mothers. Interventions that ensure normal maternal body mass index, maternal lean mass, infant size (birth and current weight) should be scaled-up to increase breast milk output and for better infant growth and body composition. Health workers should re-enforce antenatal and postnatal counselling for mothers regardless of HIV-status. Study found higher exposure to counselling by HIV-positive mothers and higher EBF rates among this group.The finding that infants of mothers on ART showed lower lean mass may need further investigation with a specific study designed to detect the variations. There is need for formulating Kenya-specific % fat prediction equations for infants. This will lead to a better understanding of the body composition of Kenyan infants in a wider scale.

CHAPTER ONE

1 INTRODUCTION

1.1 Background

Breastfeeding is an unequalled way of providing ideal nutrition for the healthy growth, development of infants (WHO 2003) and child survival. Sound breastfeeding practices such as early initiation of breastfeeding (<1 hour after birth), exclusive breastfeeding for the first 6 months of an infant age and long period of breastfeeding have been associated with positive health, nutrition and developmental outcomes including improved growth, reduced vulnerability to diseases, improved cognitive development and increased productivity later in life. As a global public health recommendation (WHO 2010), all infants irrespective of their HIV status and that of their mothers should be exclusively breastfed for the first six months of life then introduced to appropriate complementary feeding while breastfeeding is continued up to 2 years and above. Exclusive breastfeeding is the provision of breast milk alone and not providing any other solid or liquid for nourishment (WHO, 2008). Since breast milk is the only recommended source of nourishment in the first half of infancy, the amount taken is important for optimal growth and development.

Although there is paucity of studies relating to breast milk output and infections, it can be reasoned that since infections influences the maternal factors such as emotional and physical stress, they could have a major influence on breast milk output. Brown *et al*. (1986) related reduced breast milk reduction to the pre-harvest season since this is the time when food is least available. Pre-harvest season is also the apparent time when there are increased infections (Callinson, 2008), indicating that infections could have been a factor in Brown *et al*. (1986) study. Although mothers can and should continue to nurse when they have infections, infections such as sub-mastitis specifically has been found to reduce the milk output in dairy cows (Shuster *et al*., 1995). HIV exposes mothers to opportunistic infections, thus higher morbidities compared to their HIV-negative counterparts. Studies have shown higher level of subclinical mastitis in HIV-infected women (Kasonka *et al*, 2006).

1.2 HIV and Breastfeeding

Maternal HIV sero-status is one major factor that has caused a modification of the general advice on breastfeeding due to the HIV transmission risk through breast milk. The safety of breast milk from HIV-positive mothers is not fully guaranteed. The balancing act of providing the infants with the best nourishment while minimizing the risk of HIV transmission has been a real challenge. The current recommendation for mothers known to be HIV-infected (and whose infants are HIV-uninfected or of unknown HIV status) is to exclusively breastfeed for the first 6 months of life whether under antiretrovirals (ARVs) or not (WHO, 2010a). Currently there is evidence-based recommendation of provision of ARV prophylaxis to the mother or child to reduce the risk of HIV transmission during the breastfeeding period (WHO, 2010b). Part of the important reasons for the exclusive breastfeeding recommendation is the reported high mortality among young children born to HIV-positive mothers who are not exclusively breastfed (Arifen *et al*., 2001; Coovadia *et al*., 2007). As reviewed by Young *et al*. (2011), multiple studies in low-income settings have documented increased morbidity and mortality among HIV-exposed children. This has also been associated with earlier cessation of breastfeeding (Taha *et al*., 2011; Arpadi *et al*., 2009).

1.3 HIV Exposure, Breast Milk Intake and Growth

While there are recommendations and efforts to prevent mother-to-child transmission of HIV virus through breast milk, infant HIV-free survival does not represent the totality of child health and development. As reviewed by Filteau (2009) there is evidence that HIV-exposed, uninfected (HIV-EU) infants, who represent the majority of children born to HIV-infected mothers, have increased health problems. The review highlights increased vulnerability to mortality, morbidity and slower growth among HIV-EU infants compared to the HIV-unexposed infants. The review also summarizes the potential causes of poor health and nutrition among HIV-EU children as lack of parental care due to maternal death and orphan hood; infant feeding; immune abnormalities; exposure to infections and exposure to antiretroviral drugs.

Among these factors, infant feeding practice is one most modifiable factor. In the first half of infancy, breastfeeding practices are critical and influences the amount of breast milk taken. Reduced breastfeeding by HIV-infected mothers (and therefore infant breast milk intake) could account for increased infant morbidity and mortality, increased exposure to dietary pathogens, altered immune functions and growth and potentially slower development of HIV-EU infants (Filteau 2009). Despite being uninfected, HIV-EU infants show frequent growth faltering, suggesting the need for vigilance in recognizing stunting within Prevention of Mother-To-Child Transmission of HIV virus (PMTCT) programs (McGrath *et al*., 2012). Early growth in leghth and weight of HIV- exposed, uninfected (HIV-EU) infants is less than that of HIV-unexposed (HIV-U) (Masaka *et. al.*, 2007). Poor breastfeeding practices by HIV-positive mothers could be as a result of general beliefs surrounding breastfeeding in the context of HIV which could result to ultimate cessation of breastfeeding (Chisenga *et al*. 2005).

1.4 Problem Statement/Rationale for the Study

Increased efforts to scale up PMTCT services are resulting in the majority of infants being born of HIV-infected mothers being HIV-negative (HIV-EU) and their numbers will continue to increase if HIV rates do not significantly reduce. Mothers' knowledge of their own HIV status (irrespective of the HIV status of the child) may prompt them to change their breastfeeding patterns and thus affect their breast milk output. What is currently known is the age-specific normative amount of breast milk infants and young children consume (Haisma et.al, 2003; da Costa, *et al.*, 2010). It is also known that HIV exposure compromises the growth and health of uninfected infants and that one of the important pathways could be through amount of breast milk consumed. What is not known is if maternal HIV infection does affect infant breast milk intake and if this does influences early growth of infants. Paucity of this data presents a gap in better understanding the health status and growth in relation to HIV-exposure. Data on breast milk intake of HIV-EU as compared to HIV-U is important in informing further potential considerations for breastfeeding practices and infant growth in the PMTCT programs for HIV-EU. Test weighing has been for a long time been

the commonly used method to measure breast milk intake. Currently, the use of stable isotope as a method of measuring infant breast milk intake has been found to be non-invasive, not to disrupt the feeding pattern and able to detect objectively if the infant is exclusively breastfed. This study therefore primarily aimed at assessing breast milk intake among HIV-exposed, uninfected compared to HIV-unexposed infants using the deuterium oxide dose-to-mother technique. The study collected data at two points – at 6 weeks partum when the maternal body system have normally resumed (and thus stabilized milk output), and at 6 months, the cut-off time for exclusive breastfeeding.

1.5 Objectives

1. To determine the breast milk intake of HIV- exposed, uninfected and HIV-unexposed infants using dose-to-mother deuterium oxide technique at 6 weeks post-partum and at 6 months of age.
2. To determine the linear growth of HIV-exposed, uninfected infants compared to HIV-unexposed infants at 6 weeks post-partum and at 6 of age
3. To determine the body composition (lean mass) of HIV-exposed, uninfected as compared to HIV-unexposed infants at 6 months of age
4. To compare maternally reported versus deuterium oxide technique determined exclusive breastfeeding rates among HIV-positive and negative mothers at 6 weeks post-partum and at 6 months of age.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Maternal HIV Status

2.1.1 Current maternal HIV status

According to joint United Nations program on HIV and AIDS (UNAIDS), at the end of 2014, there were an estimated 36.9 million [34.3 million–41.4 million] people globally were living with HIV (UNAIDS, 2015). This represented an increase of 8.5% as compared to 2010. Of all the people infected by HIV, 68% reside in sub-Sahara Africa (Avert, 2012a). Although globally half of the HIV-infected are women, (Reproductive Rights, 2012), women are more infected in sub-Saharan Africa (59% of all people living with HIV) (UNAIDS, 2011). In Kenya, just like in many countries in sub-Sahara Africa, the HIV prevalence in women (7.6%) is higher than that of men (5.6%) of ages 15-49 years (NAAC, 2015). The prevalence in women can be as high as six times that in men among sexually active 15-19 year olds, three times than that in men among 20-24 year olds, and equal to that in men among 25-49 year olds (Glynn et. al, 2001). Biological and behavioral reasons for the differentials are well-documented (GOK, 2009; Glynn *et al*, 2001; UNAIDS, 2008).

In 2013, an estimated 1.5 million women living with HIV gave birth, virtually unchanged from 2009 (UNAIDS, 2014). HIV prevalence studies offer the most reliable data for comparing epidemics in different countries and are indicative of paediatrics HIV and AIDS (De Cock *et al*, 2000). In 2009, the prevalence of HIV among pregnant women attending Antenatal Clinic (ANC) in Kenya was 9.0% (GOK, 2007). In 2010, there were estimated between 41,000-120,000 pregnant women living with HIV in Kenya (UNICEF, 2010; USAID, 2010). Most recently, estimated HIV-exposed births per year stands at 100,500-113,900 (Muraguri, 2010).

In 2007, World Health Organization (WHO) and UNAIDS revised the guidelines for HIV testing in 2007 (WHO/UNAIDS, 2007) in which provider initiated HIV

testing was recommended. Following the adoption of this recommendation (GOK, 2010), the proportion of pregnant women tested for HIV has been on the increase and approximately 63% of pregnant women were tested for HIV in 2009 in 58% of ANC sites offering HIV-testing services (UNICEF, 2010). The guidelines on provider-initiated HIV testing were designed to increase coverage of testing and identify patients in need of antiretroviral therapy (ART). Provider-initiated HIV testing and counselling involves the health care provider specifically recommending an HIV test to patients attending health facilities. In these circumstances, once specific pre-test information has been provided, the HIV test would ordinarily be performed unless the patient declines (WHO/UNAIDS, 2007). A pregnant mother can be infected with HIV before or during pregnancy. While it is impossible for an HIV-negative woman to give birth to an HIV-positive infant, it is possible for a woman to seroconvert during her pregnancy; starting her pregnancy as HIV-negative and becoming HIV-positive through sexual transmission from a sexual partner, unscreened blood transfusions, use of contaminated injection needles or rape during the course of pregnancy (What Works for Women, 2012).

2.1.2 Effects of maternal HIV infection pre- and postnatal

Worldwide, the predominant virus is HIV-1, and generally when people refer to HIV without specifying the type of virus they mean HIV-1. The relatively uncommon HIV-2 type is concentrated in West Africa and is rarely found elsewhere (Avert, 2011). HIV-2, which is transmitted in the same ways as HIV-1, causes AIDS much more slowly than HIV-1 but clinically the diseases are very similar (Hunt, 2010).

During pregnancy, HIV increases susceptibility to opportunistic infections (OIs) such as *pneumocystis carinii* pneumonia, tuberculosis, malaria (Kumar *et al*., 1995) which can be treated by introducing ART. Seropositive pregnant women have been reported to have higher rates of many OIs as compared to the sero-negative mothers (Termarman *et al*., 1994; Tocconi, 2003; Maja *et al*., 1998). HIV infection also increases the risk of malnutrition among pregnant mothers.

Asymptomatic HIV-positive individuals require 10% more energy, and symptomatic HIV-positive individuals require 20% - 30% more energy than HIV-negative individuals of the same age, sex, and physical activity level (WHO, 2003). It then follows that a combination of increased protein and nutrient demand, and the foetal nutrient demand can reduce maternal BMI during pregnancy. Women with lower Body Mass Index (BMI) have a greater risk of perinatal HIV transmission, even after adjustments for HIV viral load and CD4 count (Banda *et al*., 2007). Micronutrient depletion among HIV-positive mothers has also been reported (Fawzi *et al*, 2005; Friss *et al*., 2001; Villamor *et al*., 2005). HIV infection affects foetal growth. Maternal HIV-1 infection is significantly associated with intrauterine growth retardation (Bulterys *et al*., 1994; Geary *et al*., 1994) and low birth weight (Kumar *et al*., 1995; Termarman *et al*., 1990; Sombie *et al*., 1994).

Chersich *et al*. (2007) found increased opportunistic infections (OIs) among HIV-infected Kenyan women during the year following delivery as compared to the HIV-uninfected women. HIV-infected women were more likely to experience fever, dyspnea, and dysuria, and to have genital warts, candidiasis, and bacterial vaginosis. Judd *et al*. (2007) also concluded that mothers with HIV-1, although generally healthy, have substantial morbidity as a result of common infections, some of which are predicted by immune status or by socioeconomic factors. About 40% of HIV–infected mothers can develop subclinical mastitis, and Na^+/K^+ ratio (which depicts subclinical mastitis) is significantly higher 14 weeks post-partum (Willumsen *et al*., 2003). Mastitis, an inflammatory process in the breast, may be common in lactating women in Africa and is associated with higher HIV load in breast milk and mother-to-child transmission of HIV (Semba *et al*. 1999). Sub clinical mastitis which is higher in HIV infected than uninfected mothers (Kasonka *et al*. 2006) has also been shown in cattle to reduce breast milk output (Shuster *et al*. 1995). However, Gomo *et al*. (2003) found out that there is no significant difference in the prevalence of subclinical mastitis between HIV - infected and the uninfected.

2.2 Transmission of HIV from Mother to Child

2.2.1 Mechanisms for vertical transmission of HIV

Vertical HIV transmission, also known as mother-to-child transmission, can occur in utero, during delivery and during breastfeeding. The precise mechanism or even route of the vertical transmission of the virus remains not well known (de Vries and Peek, 2008). However, Newell (1998) has reviewed that infected placental cells may be passed to the fetus during birth (intrauterine or transplacental transmission), direct contact of fetus/infant with infectious maternal blood and genital secretion during passage through the birth canal, amongst other avenues (intrapartum transmission) and through breast milk where cell-free penetration of mucosal lining of gastro-intestinal track of infants by infecting cells, or by direct entry into blood stream via mucosal breaches. Transmission during delivery occurs when the infant sucks, imbibes or aspirates maternal blood or cervical secretion that contain HIV or when it has other mucous membrane exposure (GOK, 2009b). The most significant risk factors for vertical transmission appear to be HIV viral load in the mother (GOK, 2009b). Infections such as subclinical mastitis among HIV-infected women may also increase the risk of vertical transmission through breastfeeding by increasing milk viral load (Willumsen, 2002).

According to De Cock *et al*. (2000), without an intervention, about 15-30% of babies born to HIV-infected women will become infected with HIV through intrauterine and intrapartum transmission. Out of those who are HIV free, 5-35% will become infected through breastfeeding in the first half of infancy. And latter, 30-45% of those who survive intrauterine, intrapartum and transmission through breastfeeding will get infected. On overall, 43.5-75% of children born of HIV-positive mothers will get infected when there is no intervention to both the mothers and children. In 2010, approximately 390,000 children under 15 years became infected with HIV, mainly through mother-to-child transmission (UNAIDS, 2011). About 90% of children living with HIV reside in sub-Saharan

Africa where, in the context of a high child mortality rate, AIDS accounts for 8% of all under-five deaths in the region (UNICEF, 2004).

2.2.2 Prevention of mother to child transmission of HIV virus

The rate of vertical HIV transmission from mother-to-child-to can be reduced to levels below 5% with effective interventions (WHO, 2012). The global community has committed itself to accelerate progress for the prevention of mother-to-child HIV transmission (PMTCT) through an initiative with the goal to eliminate new paediatric HIV infections by 2015 and improve maternal, new-born and child survival and health in the context of HIV. Already in high income countries mother-to-child transmission of HIV (MTCT) has been virtually eliminated due to effective voluntary testing and counselling, access to antiretroviral therapy, safe delivery practices, and the widespread availability and safe use of breast-milk substitutes (Avert, 2012b).

Prevention of mother-to-child transmission of HIV (PMTCT) is a dynamic and rapidly changing field. The 2010 WHO PMTCT ARV guidelines are based on the need to distinguish between treatment and prophylaxis (WHO, 2012). WHO (2012) reviews and analyses the current guidelines as follows: consistent with the 2010 WHO adult ART guidelines recommendation is to prioritize starting all women with CD4 counts ≤350 cells/ mm^3 or WHO Stage 3 or 4 disease (approximately 40–50% of all HIV-infected pregnant women) on ART for life for their own health as well as for the prevention of infant HIV infection. For women with CD4 counts >350 cells/mm^3, who are not eligible for treatment according to current criteria, the PMTCT ARV guidelines recommend starting ARV prophylaxis early in pregnancy and in breastfeeding settings, providing extended ARVs to either the mother or child during the postpartum risk period. The two recommended prophylaxis options, A and B, are quite different programmatically but were judged to be equally efficacious (in preventing the virus transmission), if implemented appropriately, in reducing the risk of infant infections for women with CD4 counts >350 cells/ mm^3. Governments are to decide which option to select. Recently, a third option, to provide lifelong ART to all HIV-infected

pregnant women, regardless of CD4 cell count, has emerged (Option B+), and a number of countries are already adopting or considering this approach (WHO 2012). Kenya has adopted the current 2010 PMTCT ARV guidelines and is in the process of updating their current guidelines (GOK, 2009b) to reflect the recent changes.

Maternal and infant HIV testing in Kenya

As explained, Kenya has adopted the provider-initiated HIV testing for pregnant mothers. In addition and in accordance to the National Guidelines for HIV testing in Kenya (GOK, 2008), testing should be offered to the parents and the child at the earliest opportunity. This may occur at an antenatal clinic (ANC), maternal and child health clinic (MCH), inpatient ward, or in any other setting. Early infant diagnosis - using DNA polymerase chain reaction (PCR) or any other appropriate technology-should be offered to all infants who are exposed to HIV. Where PCR facilities are not available, rapid tests may be used in order to determine the child's exposure status and facilitate early entry into care and treatment programmes. Attempts should be made to reach beyond the index child to other children as well.

HIV prophylactic drugs provided to HIV-positive mothers and their uninfected infants in Kenya

Kenya has adopted WHO 2012 Option A for PMTCT (Muraguri, 2010) with recommended (Antiretroviral therapy) ART regimen (GOK, 2009b) for treating pregnant women at antepartum, intrapartum and postpartum as zidovidine (AZT) (300 mg twice daily) + stavudine (3TC) (150 mg twice daily) + navirapine (NVP) (2mg/kg stat within 72 hours) daily. ART treatment is also recommended for those with CD4 less or equal to 350 cell/mm^3 or in WHO clinical stage 3 and 4 for the rest of their lives. Additionally, pregnant HIV-positive mothers should be provided with cotrimoxazole (960mg once daily) and multivitamins. Prophylaxis regimen for infants is as follows (GOK, 2009b): Single dose NVP (2mg/kg stat within 72 hours after birth), 3TC for duration of 1 week (4mg/kg twice daily) and

AZT for a duration of 6 weeks (4mg/kg twice daily). Additionally, all infants born to HIV-infected mothers, irrespective of any antiretroviral therapy during pregnancy or labour should be provided with cotrimoxazole 120g twice daily on three consecutive or alternate days in a weeks as *pneumocystis jiroveccii* pneumonia prophylaxis (PCP) until the infant is 12 months or the DNA PRC negative or antibody negative (GOK, 2009b).

2.3 HIV-Exposed, Uninfected (HIV-EU) child

2.3.1 Magnitude of the HIV-EU problem

With efforts to reduce transmission of HIV from mothers to children intrauterine, trans-placental and during breastfeeding, the majority of infants born of HIV-positive mothers will be HIV-negative. As shown by computations and assumptions in Table 1, HIV-EU infants account for a high proportion (82.1%) of all infants born of HIV-infected mothers in Kenya.

Approximately 5,930 stunted infants annually are HIV-EU and these account for about a third of all infants stunted. Increase in chronically malnourished infants means more children with limited physical, development, more vulnerable to infections and reduced cognitive development. This also means more children at greater risk of dying during their early years.

Table 2.1: Estimates of the burden of stunted HIV-EU infants in Kenya

Parameter (on per year basis except for the projections)	Kenyan estimates
Number of HIV-positive pregnant women (or expected annual HIV-exposed births)	107,200*
Number of live births born of HIV-positive mothers[μ]	103,234
Pregnant mothers accessing PMTCT- 63%[€]	65,037
PMTCT prevented with treatment - 95%[¥] (approximate annual HIV-EU)	61,785
PMTCT prevented without treatment- 60% of all HIV[Ω] (out of those who do not access the services)	22,918
Total number of HIV-EU per year	84,703
Number of HIV-EU infants as a proportion of those live births born to HIV –infected mothers	82.1%
Estimated number of stunted HIV-EU[β]	28,799
Number of stunted HIV-EU as a proportion of total infants stunted [α]	29.8

[§]UNAIDS, 2010
*Mean of the range quoted by Muraguri (2010)
[€]63% of pregnant mothers access PMTCT services in Kenya (UNICEF, 2010)
[¥] Assuming a maximum effect of 95% PMTCT (WHO, 2012)
[Ω] Assuming 40% rate of transmission from mother to child-without treatment (GOK, 2009b)
[μ]Assuming for Kenya, perinatal mortality rate as 37 per 1000 live births (GOK, 2008). This rate has been factored into the denominator.
[β]Assuming same prevalence of stunting among Malawian HIV-EU infants - 34% (Flax *et al*., 2012).
[α]Stunting among infants is 7% (GOK, 2008). Estimated birth rate is 33.54 per 1000 population. Current Kenyan population is 41,070,934 (Index Mundi, 2012).

2.3.2 Health and nutrition status of HIV –EU infants and young children

HIV-free survival does not guarantee sound health and nutrition status of infants and young children. Efforts to scale-up PMTCT lead to increase in HIV-EU infants. In the recent past, it has been shown that the health and nutrition status of HIV-EU may not be as sound as for HIV-unexposed. As reviewed by Filteau (2009) there is evidence that HIV-exposed, uninfected (HIV-EU) infants, who represent the majority of children of HIV-infected mothers, have increased health problems. The review documents vulnerability to mortality, morbidity and slower

growth among HIV-EU infants. HIV-EU infants present hematologic and immunologic abnormalities at birth (Kolte *et al*., 2011). In this study Kolte *et al*. (2011), CD4 and CD8 counts did not differ between HIV-EU and HIV-uninfected children. The incidence of Group B Streptococcal infection was significantly higher in HIV-EU infants than in infants who were born to HIV-uninfected mothers (Epalza *et al*., 2010). Response to some treatments has also been found to be poor among the HIV-EU. McNally *et al*. (2007) found that HIV-EU infants had more treatment failures for *Pneumocystis jirovecii* pneumonia than did HIV-uninfected infants.

Mortality among HIV-EU has also been found to be higher than among in the HIV-unexposed. Marinda *et. al.,* (2007) found a two-year mortality of 2.9% among the HIV-unexposed and 9.2% among HIV-EU, and the difference was significant. In another study (Shapiro *et al*., 2007), twenty-four-month mortality was 29.5% among HIV-infected infants, 6.7% among HIV-exposed uninfected infants, and 1.6% among HIV-unexposed infants. Filteau (2009) reviewed mortality among HIV-EU and –unexposed infants in 6 African countries (as reported by different studies) and found higher mortality rates in HIV-EU in all the studies.

Nutrition outcomes are also affected by maternal HIV infection. Infants born to HIV-infected women have significantly lower mean birth weight and length, regardless of the infants' HIV status, compared with infants born to uninfected women (Arpadi, 2000; Bailley *et al*., 1999; Patel *et al*., 2010; Farquhar *et al*., 2005; Lartey *et al*., 2012). Filteau *et al.* (2010) also found that children born of HIV-infected women has higher prevalence of stunting (29%) at 18 months as compared to children born of HIV-uninfected women (18%) (OR 1.80; 95% CI 1.14, 2.83; P = 0.03). By 2 years 29% of Kenyan HIV-EU children were underweight, 18% were wasted, and 58% were stunted (McGrath *et al*., 2012).

For the same age group, these rates are higher than those reported by the DHS (GOK, 2014).

Potential causes of higher mortality, poor health and nutrition among HIV-EU children are reduced immunity observed among mothers with HIV-1 (Farquhar *et al*., 2005); lack of parental care due to maternal death and ophan hood (Filteau, 2009) ; infant feeding practices (Filteau, 2009); immune abnormalities (Kolte *et al*., 2011); exposure to infections and exposure to antiretroviral drugs (Kathleen *et al*., 2011).

2.4 Breastfeeding, Infant Growth and Body Composition

2.4.1 Exclusive breastfeeding in the context of HIV

Up to half of infancy, breast milk is the only recommended food (WHO, 2003), and thus an important determinant for infant and child survival, health, growth and development. Initially the recommendation for exclusive breastfeeding was up to 3-4 months. Later, a review of then existing studies showed that the optimal exclusive breastfeeding period is 6 months (Kramer and Kakuma, 2004). A review of a wide range of published and unpublished material showed that exclusive breastfeeding for six months (versus three to four months) reduces gastrointestinal infection, does not impair growth, and helps the mother lose weight. This seemingly important review contributed to the change of the WHO recommendation on the duration of EBF to six months. A randomized controlled trial (Bhandari *et al.*, 2003) showed that promotion of exclusive breastfeeding until age 6 months in a developing country through existing primary health-care services is feasible, reduces the risk of diarrhoea, and does not lead to growth faltering.

In the HIV infection context, EBF compared to mixed feeding is associated with lower risk of postnatal vertical HIV-1 transmission (Ilif *et al*. 2005; 2005; Kuhn, 2007; Coovadia *et al*., 2007; Holmes and Savage, 2007). Breastfeeding *per se* as compared to formula feeding has no protection on mortality of HIV infected and uninfected infants (Raziya *et al*., 1997; Newel *et al*., 2004; Nduati *et al*., 2001). On the other hand, EBF is recommended because it protects infants from

morbidity and mortality whether or not HIV related (Iliff *et al*., 2005). Cessation of breastfeeding among HIV-EU is associated with acute morbidity events and cumulative mortality (Taha *et al*., 2011; Fawzy *et al*., 2009). For infants, breastfeeding is unequalled in its role in child growth (Young *et al*., 2011). Growth falters among the HIV-uninfected children born of HIV-positive mothers if breastfeeding is stopped (Arpadi *et al*., 2009). EBF is an affordable, feasible, acceptable, safe and sustainable practice that also reduces HIV transmission providing HIV-infected women with a means to protect their children's lives (Kuhn, 2007).

WHO 2010 guidelines on HIV and infant feeding (WHO, 2010) are based on the evidence that antiretroviral (ARV) interventions to either the HIV-infected mother or HIV-exposed infant can significantly reduce the risk of postnatal transmission of HIV through breastfeeding. These revisions to the 2006 guidelines (WHO *et al*., 2007) recommend that national authorities in each country decide which infant feeding practice, i.e. breastfeeding with an antiretroviral intervention to reduce transmission or avoidance of all breastfeeding, will be primarily promoted and supported by Maternal and Child Health services. The ARVs provided to mothers prevents the opportunistic infections. This coupled with psychosocial and health counselling improved the mothers' health and well-being, which improves the propensity of the mother to care for the infants and young children.

Kenya has increased efforts to upscale PMTCT services through mandatory HIV testing and counselling of pregnant women. At the same time, Kenya has adopted EBF as a national policy for feeding of infants below six months (GOK, 2012). The policy clearly indicates that all HIV-positive pregnant women should be evaluated for Highly Active Antiretroviral Therapy (HAART) eligibility and if not eligible, provided with ARV prophylaxis. HIV-exposed infants should receive appropriate ART prophylaxis according to national guidelines (GOK, 2010b). Specifically on infant feeding for HIV-exposed infant:

1. Mothers known to be HIV-infected (and whose infants are HIV-uninfected or of unknown HIV status) should exclusively breastfeed their infants for the first 6 months of life, introducing appropriate complementary foods after 6 months, and continue breast-feeding two years and beyond. Both mother and their infants should receive prophylaxis or anti-retroviral treatment in line with the national recommendations.
2. HIV-positive women (and whose infants are HIV-uninfected or of unknown HIV status), who choose not to breastfeed, should be given information on the special conditions of Acceptable Feasible Affordable Sustainable and Safe (AFASS) that should be met. If these conditions are met, she should be counselled and supported to do exclusive replacement feeding using infant formula for the first 6 months and appropriate complementary feeds introduced at 6 months. Infants of these mothers should be provided with appropriate antiretroviral treatment.

In Kenya, health workers are therefore expected to test all pregnant women for HIV, counsel them and provide HIV treatment, PMTCT services and promote EBF.

2.4.2 Exclusive breastfeeding and breast milk output

Exclusive breastfeeding establishes regularity of breast milk supply and demand. During exclusive breastfeeding, milk intakes are high and increase over time, and there is adequate energy intake, normal infant growth, and no marked changes in breastfeeding practices (Nielsen *et al*., 2011). In this prospective study to investigate the adequacy of milk production among mothers in greater Glasgow (Scotland) during exclusive breastfeeding (Nielsen *et al*., 2011), milk intakes were higher than literature values (923 [SD:122] g/day, n = 36; and 999 [SD: 146] g/day, n =33) at both 15 and 25 weeks of age respectively (both P<.001) and increased significantly between time points (mean increase: 61 g/day [95% confidence interval: 23–99]; P= .003). This study used the doubly labeled water method. In a comparison study using dose-to-mother deuterium-oxide technique (Wells *et al*., 2012), breast-milk intake among Iceland infants was 83 g/d (95%

CI: 19, 148 g/d) greater (and significantly different) in EBF group (mean ± SD: 901 ± 158 g/d) than in complementary feeding group (818 ± 166 g/d) infants.

Despite its potential positive health and nutrition implications, EBF is still uncommon in most communities. The prevalence of EBF in developing countries and in Africa is 43% and 46% respectively (UNICEF, 2011). In Kenya, EBF prevalence stands at 32% (GOK, 2014). EBF is easily undermined not only by the marketing efforts of formula manufacturers, but also by a wide range of traditional and modern cultural beliefs and poor health-care practices (Holmes, 2007).

In sub-Sahara Africa, breastfeeding among mothers who are HIV-positive has raised public health concerns over HIV transmission. In many parts of sub-Saharan Africa messages on the role of breastfeeding in HIV-transmission are still not clear. EBF among HIV-positive women in this region may still be low. Among HIV-positive mothers, 37% of women supported for good breastfeeding practice were still exclusively breastfeeding at week 16 postpartum (Chisenga *et al*., 2005). Among the HIV-EU infants, EBF rates have been found to be substantially low (<5%) (Mc Gath 2011). Factors that have beeen associated with shorter duration of EBF among Zambian HIV-positive mothers are primiparity, maternal systemic illness, and infant length at 6 weeks (Chisenga *et al*., 2005).

2.4.3 Factors affecting breast milk output/lactation capacity

The mechanism of milk production and release in humans is described by Riordarn (2005). Highly vascularized secretory cells in the mammary glands extract water, lactose, amino acids, fats, vitamins, minerals and numerous other substances from the mothers blood converting them to milk from her infant. Stores of adipose tissue laid down during pregnancy are drawn upon to provide milk synthesis.

2.4.3.1 Maternal factors affecting breast milk output

Various maternal factors have been known to affect human milk output. Among the maternal various factors affecting breast milk output, parity is a key one (Whitehead *et al*., 1978). Parity was found to be the most significant factor affecting breast milk volume at 1 week postpartum (multiparous women delivered 142 ml more milk in 24 h than primiparous women (Ingram *et al*., 1999). Acute physical and mental stress can impair the milk ejection reflex by reducing the release of oxytocin during a feed and if this occurs repeatedly, it could reduce milk production by preventing full emptying of the breast at each feed (Dewey, 2001; Infante *et al*. 1985). Sources of stress can be those imposed by preterm delivery, infant medical condition, or maternal lifestyle (Lau, 2001). Depressive symptoms early in the postpartum period may lower the prevalence of breastfeeding (Harton *et al*., 2005) and thus the breast milk output. In another related relationship, breastfeeding is associated with enhanced physical and mental health compared to non-breastfeeding (Mezzacappa, 2004) - compared with not breastfeeding, breastfeeding is associated with increased parasympathetic nervous system modulation, greater vascular stress response, lower perceived stress levels, and fewer depressive symptoms. The causality between breastfeeding and these mental health indicators are however far from been proven. Although mothers can and should continue to nurse when they have infections, infections such mastitis has been found to reduce the milk output in dairy cows (Shuster *et al*. 1995). There is paucity of studies relating breast milk intake and infections. HIV exposes mothers to opportunistic diseases, thus higher morbidities as generally compared to their HIV-negative counterparts. However, Peruvian maternal infections (acute febrile infection) as indicated by elevated levels of C-reactive protein in maternal serum during established lactation did not affect milk volume as it was found by Zavaleta *et al*. (1995). The milk output was however, assessed by the 12-h test-weighing method which affects maternal behaviour and infant appetite, resulting in unrepresentative data (ICH, 2010).

Institute of Medicine (IOM) has found that although animal studies illustrate that milk yield is decreased by dietary restriction, findings from energy supplementation studies in humans are not conclusive (IOM, 1991). Earlier studies have also shown a natural maternal response when food intake is limited. The prolonged high prolactin concentrations found in undernourished mothers may ensure milk synthesis when food intake is limited, by preferentially channeling nutrients towards the breast (Lunn *et al.* 1980). However, in some old studies in Kenya, nutrition status appeared to influence breast milk output (Van Steenbergen *et al*. 1983). Relationships between milk output and parity had also been thought to be mediated through maternal nutritional status (Infante *et al*., 1985). Physical activity can also influence breast milk production.

2.4.3.2 Infant factors affecting breast milk intake

A number of infant factors affecting breast milk intake have been documented. Gestational age appears to affect breast milk output. Mothers of preterm infants 6 weeks postpartum were 2.81 times more at risk of not producing adequately (defined as = 500 mL/d at week 6) than mothers of term infants (Hill *et al*., 2005). Some early studies in Kenya indicated that male infants suckle more than their female counterparts resulting in the difference in milk output (Van Steenbergen *et al*. 1983). Peak intake of males is higher and is achieved faster (3 months) than among girls (four months) (Paul *et al*., 1988). Social-cultural factors could be involved as well. In some societies, son preference may lead to discriminatory practices against females (Ren, 1995) and increased nutrition investments in the males (Powe *et al*., 2010). Infants with higher weight –for-age have increased breast milk intake (Whitehead *et al*., 1978). Infant birth weight, weight at 3 months, and total time nursing have been found to be positively associated with breast milk intake (Dewey *et al*., 2001). It has also been found that a mother's milk production is likely to be a reflection of her infant's appetite, rather than her ability to produce milk (Daly *et al*., 1995; Dewey and Lonnerdal, 1986). Kent *et al*. (2006) suggests that breastfed infants should be encouraged to feed on

demand, day and night to increase milk production. Dewey *et al*., 2001 found that infant demand was the main determinant of lactation performance.

One of the main reasons for encouragement of breastfeeding among infants (including exclusive breastfeeding in the first half of infancy) is to encourage better growth. Studies have shown that infants who are have higher breast milk intake grow better than those who take less breast milk. In the early months, the infants' weight accounted for a major part of the variance in breast milk intake (Paul *et al*., 1988). Among Peruvian infants, it was found that there were no changes with illness in the frequency of breast-feeding, total suckling time, or amount of breast-milk energy consumed (Brown *et al*.,1990).

2.4.4 Breast milk intake and growth among infants

Breast milk intake is measured by weight (weight of breast milk/day or weight of breast milk/child weight/day) or by volume (volume of breast milk/day). Breast milk intake differs by age of the infant. In a pooled analysis of 1,115 data points of human milk intake of children 0-24 months old by dose-to-mother deuterium oxide technique, da Costa *et al*. (2010) report the overall mean intake of 0.78 (95% CI = 0.72, 0.84) kg/d. The intake increased over the first 3–4 months and remained above 0.80 kg/d until 6–7 months of infancy. The variability of intake increased in late infancy. Boys consumed 0.05 kg/d more than girls ($P < 0.01$). One modifiable factor that influences growth and development of infants is breastfeeding practices and by extension breast milk intake. Although exclusive breastfeeding is recommended for children below 6 months irrespective of HIV infection, maternal HIV infection may lead to alteration of breastfeeding practices which in turn may lead to altered breast milk intake. HIV-infected mothers in developed countries almost universally do not breastfeed their infants because of a ~15% risk of HIV infection being transmitted in breast milk (Shearer, 2008). HIV-positive mothers in resource-constrained settings may be so motivated to protect their child from HIV that they stop breast-feeding earlier than those who are HIV-positive or those of unknown status (Lunney, 2008; Chisenga *et al*., 2010). One major implication of altered breastfeeding practices due to maternal

HIV infection is on infant growth. Reduced infant growth in the first year of life is associated with maternal HIV status (Lartey *et al*., 2012). Risk of decline in length for age to <2 Z scores is associated with increasingly lower maternal age, lower maternal CD4 cell count, premature birth and formula feeding (Vanketesh *et al*. 2010). Growth faltering of infants and cessation of breastfeeding due to HIV are directly related as depicted by a study on HIV- uninfected Zambian children 4.5-15 months old born to HIV-positive mothers in Zambia (Arpardi *et al*., 2009). It has been shown that at birth, there is no significant difference in length-for-age among HIV-infected infants, HIV-EU and HIV-unexposed (Bailey *et al*.1999). However, HIV-infected infants are more likely to be stunted and wasted than their HIV-EU and HIV-unexposed counterparts at 3 months after birth (Vankatesh *et al*., 2010; Bailey *et al*.1999; Lepage *et al*., 1996).

2.4.5 Body composition and associated factors

Body composition is the proportion of water, fat, bone and muscles. The human body is often subdivided into two component parts in a two compartment model: fat mass (FM) and fat free mass (FFM) (IAEA, 2009 or is it 2009). Body composition is an important measurement used as an indicator for 'optimal' growth in infants and children. Weight gain alone lacks information about whether the child is mainly gaining lean (fat-free) or fat mass. However, the assessment of growth during this crucial period of early vulnerability is largely based on anthropometric measurements such as body weight, with insufficient attention to the "quality of growth" and the relative partitioning of nutrients to fat free mass (FFM) or fat mass (FM) (IAEA, 2009). In order to better understand the associations between growth during early life and later health status, there is an urgent need to better capture the dynamic nature of growth during early life by assessment of body composition i.e., the partitioning of FM and FFM (Ahmad *et al*., 2010).

The body composition in early life has significant health effects in the future life health outcome including obesity, hypertension, type 2 diabetes, cardiovascular diseases and stroke. In various locations and settings, body compositions of

infants have been reported including among the Srilankan infants (Bandara, 2015), United States of America (USA) infants (Field *et al*, 2011) and for infants from different sources (Fomon *et al*., 1982). Factors that affect body composition (specifically infant lean mass) has been associated is associated with maternal height (Harvey *et al*., 2005) and maternal BMI (Hull *et al*., 2008). Infant factors associated with the lean mass include birth weight (Wells *et al*., 2007) and gestation age (Lapillonne *et al*., 2008). While no indication of published data comparing the amount of breast milk intake and body composition, there are a number of studies that associate infant feeding practices and body composition. For instance, the multicomponent model indicated that FFM was lower in BF than FF infants at 3 months, and FM and %FM were higher in BF than FF infants at 3 and 6 months (Butte *et al*., 2000). Further, while the body composition differences between the HIV positive infants and HIV-negative infants are known, there is no indication of body composition comparison between uninfected infants born of HIV- positive and negative mothers. Lean body mass of HIV-positive infant has been found to be lower than in an HIV-negative comparison group at early stages of HIV infection.

2.5 The Causal Analysis for Maternal HIV Infection, Breast Milk Intake and Growth of Infants

Figure 2.1 shows the conceptual causal analysis for HIV infection, breast milk intake and early growth of infants based on the reviewed literature. HIV infection affects the breast milk intake and growth of infants through a number of pathways.

1. ART pathway: As soon as pregnant mothers are diagnosed of HIV, they are put on ART. The ART are passed to the infants intrauterine and /or during breastfeeding. This causes immune abnormalities and other negative effects which may lead to poor infant growth.
2. Maternal health pathway: Opportunistic infections among HIV-positive mothers means the infants are exposed to infections which may be passed on to them. This may directly lead to reduced breast

milk intake poor growth. Infections such as subclinical mastitis may also reduce breast milk output.

3. Maternal mortality pathway: The absence of the mother due to maternal death means that the infant would not have access to breast milk.
4. Maternal psychosocial status pathway: Unsound psychosocial status lead to poor breast milk output.
5. Infant low birth weight pathway: Infants who are bone with birth weight of <2500g have higher likelihood of taking less breast milk. They also gain weight and grow poorly.

There are maternal and infant factors (as identified in literature) that affect the pathways as confounding factors for HIV-infections, breast milk intake and growth as shown in Figure 2.1.

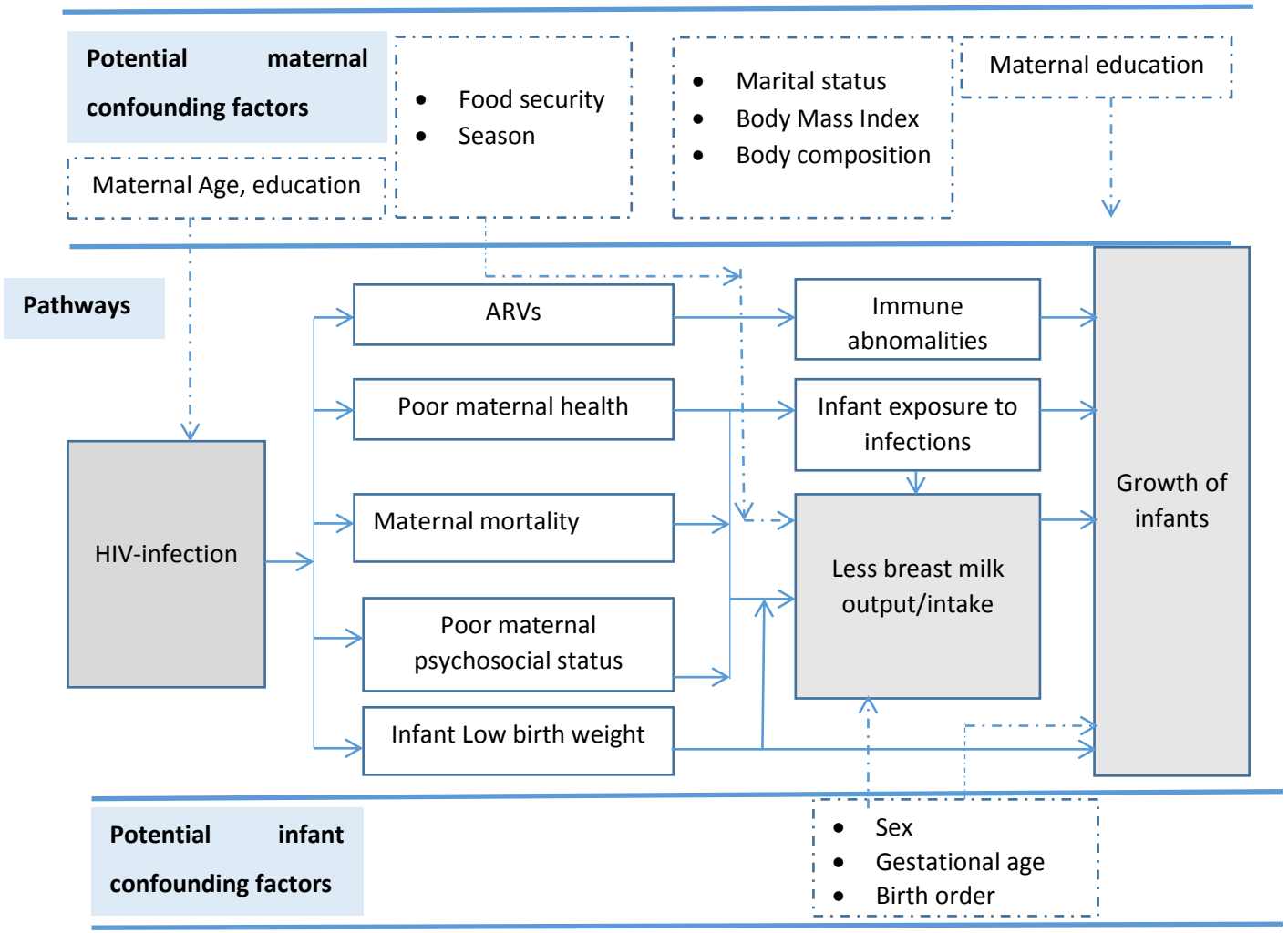

Figure 2.1: Pathway for HIV-infection affecting breast milk intake and growth of infants below 6 months old

2.6 Breast Milk Intake Determination

The measurement of breast milk intake has evolved over years. The traditional method is test-weighing, which involves weighing the baby before and after each breast feed in order to calculate the weight gain attributable to milk intake during the feed. This approach is being replaced by the use of stable isotope technique because of its shortcomings including:

1. Disturbs both maternal behaviour and infant appetite, resulting in unrepresentative data (ICH, 2010)
2. The technique is time consuming apart from disturbing the normal feeding (Savenije and Brand, 2006)
3. In many settings, infants are nursed frequently, on demand, including during the night, which results in practical limitations to the use of test weighing (IAEA, 2010). The technique is thus invasive if the study enumerators have to spend overnights in the study subject houses.

The practical limitations and the need to improve on accuracy of measuring the breast milk intake has been improved by use of stable isotope technique, which is non-invasive and can also indicate whether the child in exclusively breastfed or not. In this method, the amount of human milk consumed by the baby over a period of 14 days is assessed using the deuterium oxide 'dose-to-mother' technique, which involves giving the mother a drink of deuterium labelled water and following the disappearance of the deuterium from the mother and its appearance in the baby (IAEA, 2010).

As explained by IAEA (2010), deuterium is a stable (non-radioactive) isotope of hydrogen with the symbol 2H. It is given orally as deuterium oxide (2H2 O) and after mixing with body water is eliminated from the body in urine, saliva, sweat and human milk. Deuterium oxide is metabolized in the body in the same way as water, and is dispersed through the body water within a matter of hours. Body water can be

sampled in the form of saliva, urine, plasma or human milk and the enrichment of deuterium can be measured by Isotope Ratio Mass Spectrometry (IRMS) or Fourier Transform Infrared Spectrometry (FTIR). FTIR is not suitable for analysis of urine or human milk samples. The technique is not as sensitive as IRMS and, therefore, a larger dose of deuterium oxide is required. However, FTIR instrumentation is easier to use and maintain than that of IRMS, is less expensive to buy and the cost of analysis is lower. FTIR is therefore, particularly suitable in settings where resources are limited. In the use of saliva as the body fluid to detect deuterium, mother is dosed with deuterium oxide (by drinking). Deuterium appears in mother fluids including in the breast milk.

As the baby breastfeeds, the baby consumes the deuterium. The deuterium oxide is then detected in both mother and baby saliva over 14 day period (Days 1, 2, 3, 4, 13 and 14) using FTIR. Using the data generated the disappearance of the deuterium in the mother and appearance in the child's saliva can be depicted as shown in Figure 2.2.

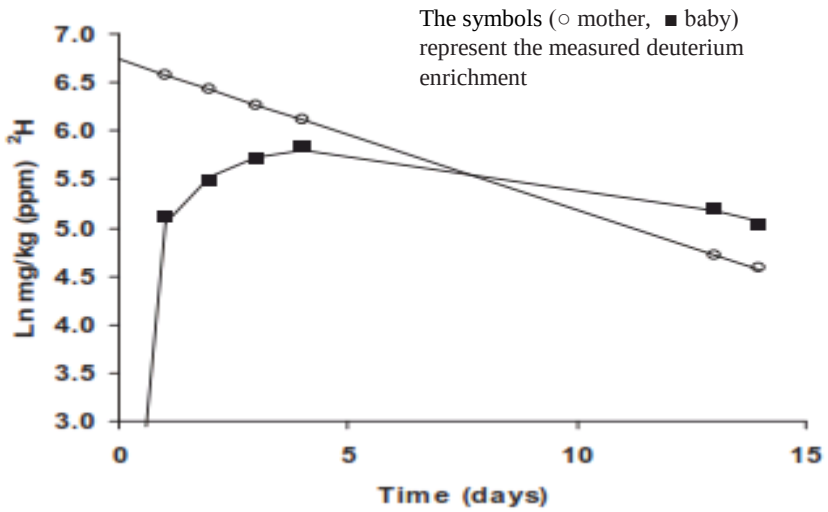

Figure 2.2: Deuterium enrichment in the body water of a mother and her baby (IAEA, 2010)

Using the mathematical models shown in section 3.6.3 (in chapter 3), the following are computed with the aid of a spread sheet developed by IAEA (2010):

1. Breast milk intake of the baby;
2. Baby total intake of water from other sources other than human milk (includes water from oxidation of milk solids and water from sources other than human milk) ; and
3. Oral water intake from sources other than human milk. The last two indicate exclusive breastfeeding in the first half of infancy.

The amount of deuterium consumed in studies of human milk output and body composition enriches the body water to a maximum in the region of 0.1% (IAEA, 2010). The threshold of deuterium toxicity has been defined as 15% and is far in excess of concentration conceived for use in human studies (Jones and Leatherdale, 1991).

2.7 Body Composition Determination Using Stable Isotope Method

Water is the largest component of the body. At birth, the body contains 70–75% water, but as the body matures, this proportion decreases to 50–60% in lean adults and to less than 40% in obese adults (IAEA, 2009). Water is found exclusively within the FFM, which is approximately 73.2% water in adults. Total body water (TBW) includes both intracellular fluid and extracellular fluid. With an estimate of TBW, the amount of FFM can be estimated. Body FM is the difference between body weight and FFM.

Given the necessity for an international consensus, the International Atomic Energy Agency (IAEA) initiated a review of body composition assessment techniques in 2009 as the basis for efforts toward the standardization of body composition assessment from birth to 2 years of age. In this method, as described by IAEA (2011) children are given a fixed standardized dose of deuterium labelled water depending on their body weight. A saliva sample is then taken before and three hours after the

dose and assessed for deuterium enrichment using Fourier transform infrared (FTIR) spectrophotometry. Deuterium concentration post dose are used to estimate of total body water (TBW). If the body weight and total body water are measured and the hydration of the fat-free mass is known, then the body's fat mass can be calculated as:

$$\text{Fat} = Wt - \text{TBW}/h_{\text{FFM}}$$

Where:
Wt is weight of the child
TBW is the Total body water
h_{FFM} is the hydration of free fat mass (hydration factor)

The hydration factors are age and sex specific and are available as estimated by Fomon *et al*. (1982). At 6 weeks after birth (~ 2 months), the hydration factor for boys is 80.3% and for girls stands at 80.2%. At 6 months of age of infants, the hydration factors are 79.6% and 79.3% for boys and girls respectively. The hydration factor for 6 months infants is generally thus considered as 79% for both sexes.

CHAPTER THREE

3 GENERAL METHODS

3.1 Study Design

This was a prospective cohort study with cross sectional data collection at 6 weeks and 6 months post-partum in which breast milk intake, growth and body composition of HIV-uninfected infants born of HIV-positive (study group) and HIV-negative mothers (control) were measured and compared at 6 weeks post-partum and at 6 months after birth. Infant feeding practices were also assessed at the two time points. Breast milk and body composition were measured using stable isotope technique (deuterium oxide dose-to-mother technique).

3.1 Study Setting

The study was set in a Maternal and Child Health (MCH) Clinic of Siaya County Referral Hospital in Western Kenya. Mother-infant dyads were recruited into the study as they attended post-natal care between February 2014 and September 2014. Siaya County is in the Lake Region of Kenya where HIV rates are highest. The county which is about 60 km from Kisumu City is inhabited by predominantly rural population with a HIV prevalence of 15.9% among adult men and 23.3% among adult women. These rates are way above the national average of 5.6% and 7.6% among adult men and women respectively (NAAC, 2015).

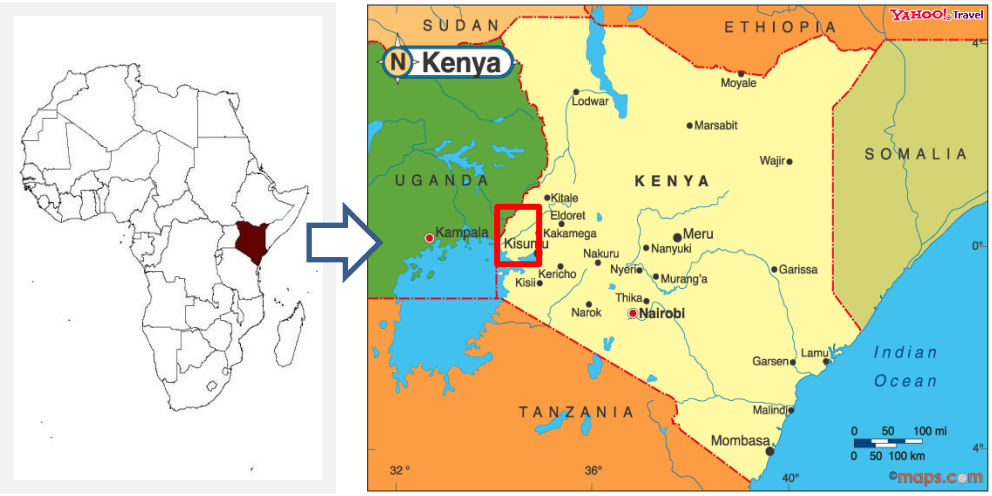

Figure 3.1: Location of Siaya County

3.2 Sample Size Determination

The sample size was computed based on the aim of comparing breast milk intake of HIV-uninfected infant born of HIV-1 positive and negative mothers using a statistical approach by Kirkwood and Sterne (2003) as shown in the equation 1.

$$\frac{(u+v)^2(\delta_1^2+\delta_0^2)}{(\mu_1-\mu_0)^2} \quad \text{..... Equation 1}$$

Where

u= One sided % point of normal distribution corresponding to 100%- the power=1,52;

v=% point of the normal distribution corresponding to two sided significant level=1.28;

$(\mu_1 - \mu_0)$= Expected difference in means; and

δ1, δ0= Standard deviations (s.d).

A mean difference of 10g/kg body wt/day in breast milk intake between HIV-EU and HIV-U infants and Standard Deviation (SD) of 18 g/kg body wt/day was adopted (Galpin *et al.*, 2007). With a statistical power of 80% and 5% level of significance, a minimum sample size of 50 mother-infants dyads for each of the two age cohorts was required. With an attrition rate of 20%, a minimum sample size of 60 for each group was arrived at. Only infants whose breast milk data was collected at both 6 months and 6 weeks were used for the analysis. These were 60 HIV-positive mothers/HIV-EU infant dyads and 62 HIV-negative mothers/HIV-U infant dyads as shown in the study profile in Figure 3.2.

3.3 Subject Recruitment and Follow-up

Mothers coming for first postnatal clinic for gynaecological check-up and/or for infant vaccinations were systematically sampled. Using the hospital registers, it was estimated that on a busy day of the week, about 20 mother-infant dyads were expected at the postnatal clinic. The target was to consent half of post-natal attendance each day. Alternate mothers were considered for the study. The first mother to line up for the antenatal care was recruited and second was not considered unless the preceding mother declined to participate in the study. The process was

repeated until the end of each working day at the MCH. The MCH was operational from Monday to Friday every week except on the official Kenyan holidays.

The study profile is a shown in the Figure 3.2. Seventy five (75) HIV-positive and 68 HIV-negative mothers-infants were recruited when the infants were 6 weeks old.

For HIV-positive mothers: Seven HIV-positive mothers and their infants were lost during the 2 weeks follow-up for DO technique measurements at 6 weeks postpartum. Four (4) of them did not turn up at when the infants were 6 months old. Two infants were excluded from the 6 months analysis due to sero-convertion. Two mothers-infant dyads did not complete the 2 weeks follow-up for DO technique measurements at 6 months.

For HIV-negative mothers: 1 mother-infant dyad was lost during the 2 weeks follow-up for DO technique measurements at 6 weeks. Four dyads did not turn up at 6 months follow-up and 1 did not complete the 2 weeks follow-up for DO technique measurements at 6 months.

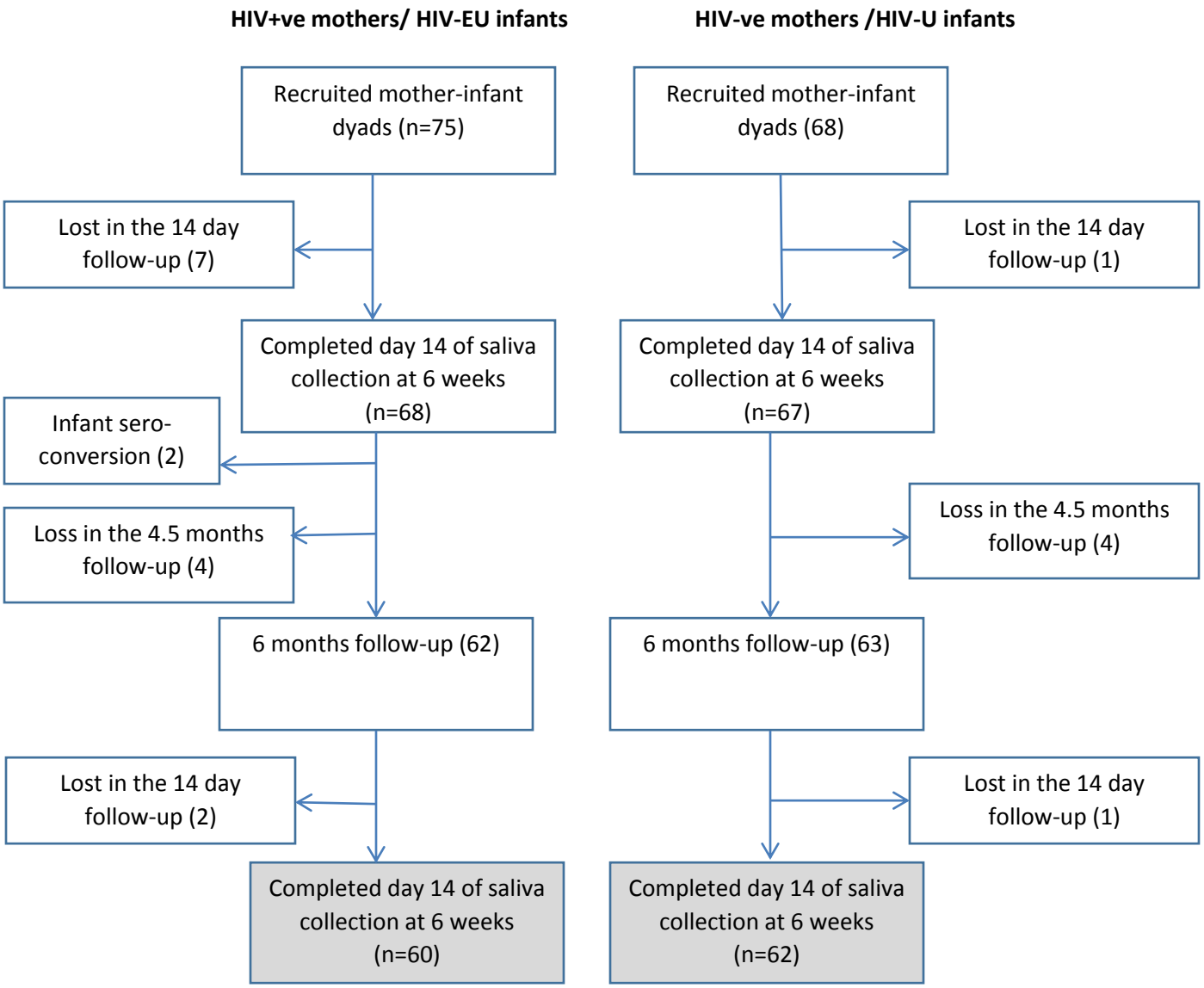

Figure 3.2: Study profile

3.4 Inclusion and Exclusion Criteria

Mother-infants dyads of 6 weeks old HIV-uninfected infants were eligible for this study. The following mothers-infant dyads were excluded from the study: those with infants having <2500g birth weight, with preterm infants, those not able to breastfeed and mothers or infants showing signs of chronic illnesses. To minimise loss to follow-up, those who intended to move far away from their then current locales 6 months from the time of recruitment were also excluded

3.5 Maternal and Infant HIV testing

Maternal HIV status at 6 weeks partum and 6 months after birth was determined by antibody testing by Colloidal Gold, (KHB Shanghai Kehua Bioengineering Co. Ltd). At 6 weeks of age, infants were tested with HIV-1 DNA polymerase chain reaction (PCR) using T100 Thermal Cycler (Bio-Rad Laboratories Inc, UK). It was not possible to test the HIV-EU infants at 6 months of age because of the Kenya government guidelines on HIV infants which stipulates that the test should be done when the infants are 9 months old using an antibody test and confirmed with PCR (GOK, 2012). The HIV-EU infant's HIV tests were thus done by 9 months to confirm if the infants were still HIV-negative. Two HIV-EU infants seroconverted at 9 months and were thus excluded from the study.

3.6 Data Collected and Methods

3.6.1 Breastfeeding practices and socio-demographic characteristics

Using a structured questionnaire, data on socio-demographic and economic status, timely initiation of breastfeeding, exclusive breastfeeding, expression of breast milk, counselling on breastfeeding were collected by recall at recruitment (6 weeks postpartum) and at follow-up (6 months of age of infants). WHO definitions of breastfeeding practices were adopted (WHO, 2008). Timely initiation of breastfeeding was defined as putting infants to breast within one hour of birth. Exclusive breastfeeding was defined as feeding infants solely on breast 24-hours preceding the interview. Standard DHS question of recalling 24 hour infant intakes of the following foods was adopted: breast milk, plain water, sugar or glucose water, gripe water, sugar-salt-water solution, fruit juice, infant formula, tea infusion, honey, milk, porridge and honey. Gestational age of the infants, date

of birth and birth weight were obtained from the mother and child clinic cards, and confirmed by mother recall. The study clinic based the determination of the gestational age on mother's last reported menstrual period. Maternal and infant infections recall data was based on the observed sicknesses two weeks prior the interview.

3.6.2 Growth

Anthropometric measurements were taken using the Standard Operation Procedures described in the Appendix 3.1.

Height/length: Mother height and infant length was determined using the Infant/Child/Adult ShorrBoard (79" x 1/8" / 200 x 0.1 cm) measured to the nearest 0.1 cm.

Weight: Maternal weight was determined by using Camry weighing scale (Model EB 9318), and recorded to the nearest 0.1 kg, while the nude weight of infants was measured using the Salter Electronic Baby (maximum 25kg).

MUAC: ShorrTape (65 cm x 0.1 cm) and Shorr Child MUAC Tape (26 cm x 0.1 cm) were used for determining the maternal and infant MUAC respectively.

3.6.3 Determination of breast milk intake

3.6.3.1 Saliva samples collection and analysis

After being weighed, baseline saliva samples were collected from the mother and the infant (day 0- T_o) using a sterile cotton wool and syringe. Mother were then be given 30g of deuterium oxide ($^2H_2$ O) through a straw. They were then instructed to feed the infant as usual and allowed to go home. At least 2ml of saliva samples were subsequently be collected from mother and infant in 10ml polypropylene sterile tubes on days 1, 2, 3, 4, 13 and 14 (corresponding toT_1, T_2, T_3, T_4, T_{13} and-T_{14}). It was ensured that post-dose saliva collection is done at the same time as time for baseline saliva collection on day 0 (T_o). All labelled tubes polypropylene sterile tubes were separately secured in zip-lock polythene bags and immediately frozen in -20oC freezers in Siaya County Referral Hospital. Samples were then

transported in cool boxes for to Kenya Medical Research Institute (KEMRI) nutrition laboratory in Nairobi, Kenya. Deuterium enrichment in mother/infant saliva over a 14-day period was measured against a standard in the Fourier Transform Infrared Spectrophotometer (FTIR 8400 Series, Shimadzu Corporation, Kyoto, Japan) at KEMRI nutrition laboratory. Human milk intake and intake of water from other sources other than breast milk was then calculated using a spread sheet developed by IAEA (IAEA, 2010). The spread sheet also computes Total Body Water which is used to estimate the maternal body composition. These maternal body composition values have also been reported.

3.6.3.2 Estimation of human breast milk intake

A MS-Excel^{TM} spreadsheet has been developed by IAEA to compute the amount of breast milk intake from the deuterium enrichment data (IAEA, 2010) as explained briefly hereby. Intake of human milk and water from sources other than human milk were then calculated by fitting the deuterium enrichment data to a model for water turnover in the mother and in the baby.

In the steady state, water turnover in the mother is given by a single exponential equation:

$$\frac{E_{m(t)}}{E_{m(0)}} = e^{-k_{mm}t}$$

…….. Equation 2

Where: $E_{m(t)}$ is the deuterium enrichment in the mother's body water at time t, in mg/kg or ppm; t is the time since the dose was taken, i.e. time post-dose in days; $E_{m\,(o)}$ is the deuterium enrichment in the mother's body water at time zero mg/kg (ppm), i.e. the y intercept of the isotope elimination curve (log/linear plot of enrichment of ^{2}H in body water versus time); K_{mm} is the fractional water turnover in the mother (kg/d), i.e. the gradient of the isotope elimination curve .

Data from the baby are fitted to the following multi-exponential model:

$$E_{b(t)} = E_{m(0)}\left(\frac{F_{bm}}{V_b}\right)\left(\frac{e^{-k_{mm}t} - e^{-(F_{bb}/V_b)t}}{(F_{bb}/V_b) - k_{mm}}\right)$$ …….. Equation 3

Where: $E_{b(t)}$ is the deuterium enrichment in the baby's body water at time, t, in mg/kg(ppm); t is the time since the dose was taken by the mother, i.e. time post-dose in days;0); $E_{m(o)}$ is the deuterium enrichment in the mother's body water at time zero mg/kg (ppm), i.e. the y intercept of the mother's isotope elimination curve (log/linear plot of enrichment of ^2H in the mother's body water versus time); F_{bm} is the transfer of water from the mother to the baby via human milk (kg/d); V_b is the baby's total ^2H distribution space (kg). $V_b$is assumed to change linearly with initial and final values determined from the baby's weight (W,kg). Vb = 0.84 W0.82; k_{mm} Is the fractional water turnover in the mother (kg/d), i.e. the gradient of the mother's isotope elimination curve (Figure 1); is the total water loss in the baby (kg/d). Curve fitting can be performed using the 'solver' function in MS-Excel^{TM} as explained by IAEA (2010). This procedure requires initial estimates for the unknown parameters ($C_{m(0)}$, F_{bm}, k_{mm} and F_{bb}) and, subsequently, refines them to converge on best fit values. Maternal body water volume (V_m) can be calculated from the dose given, and C and maternal water intake can be estimated as:

$$F_{mo} = V_m \times k_{mm}$$ …….. Equation 4

Calculation of M: human milk intake by the baby: Human milk intake by the baby is calculated from the flow of water from the mother to the baby, assuming that human milk is 87.1% water. M = F_{bm}/0.871 kg/d, measured human milk intake is often expressed as g/d. …….. Equation 5

Calculation of Fs: the baby's water intake from sources other than human milk: The baby's total intake of water includes water from the oxidation of milk solids (protein, fat and carbohydrate) and water from sources other than human milk.

The total water input derived from human milk is F_m. Calculation of F_s assumes that water input equals water output. Allowance must be made for the baby's growth (F_g) and for an increase in TBW during the two weeks of saliva sampling and the fact that water lost in the baby's breath and by transdermal evaporation (F_{ob}) is subject to isotopic fractionation, and for absorption of atmospheric water by the skin, mainly in the lungs (F_a).

Water input = (F).Water input ($F_m + F_a + Fs$..….. Equation 6

Water input equals water output plus water from growth ($F_{ob} + F_g$); therefore:

$F_s = F_{ob} + F_g - F_m - F_a$. …….. Equation 7

Calculation of total water input to the baby derived from human milk: The flow of water from the mother to the baby (F bm) represents free water in milk and does not include water from the oxidation of milk solids (protein, fat and carbohydrate): Human milk is assumed to contain 87.1% water, 1.3% protein, 4.1% fat and 7.2% carbohydrate; The yield of water from 1 g of protein is 0.41 g, from 1 g of fat 1.07 g and from 1 g of carbohydrate 0.55 g. Therefore, oxidation of milk solids gives about 9 g of water per 100 g of human milk. Total water input to the baby derived from human milk (F_m) is given by:

$Fm = F_{bm} + 0.09M$…….. Equation 8

Adjustments for baby's growth (F_g); Growth of the baby during the experimental period will result in a small change in the baby's deuterium distribution space, which is related to its TBW, and in this context is known as Vb . Vb is assumed to change linearly with initial and final values determined from the baby's weight (W, kg). $Vb = 0.84\ W^{0.82}$. Water gained during the experimental period, Fg is given by:

$$F_g = (V_{b,\ day14} - V_{b,\ day0})/14$$ …….. Equation 9

Adjustments for isotopic fractionation: Deuterium is lost from body water via breath and insensible routes via the skin (transdermal evaporation) more slowly than hydrogen, for the reasons described above; therefore, F_{bb} must be corrected for isotopic fractionation. Total water output from the baby, i.e. flow from the baby to the outside F_{ob}), which includes water lost as urine, sweat, in faeces and in breath, includes a correction for isotopic fractionation. The isotopic fractionation factor for deuterium between water vapour and water liquid is 0.946 at 37ºC. It is assumed that 85% of the baby's water output is not fractionated and that the remaining 15% is fractionated by a factor of 0.946. Thus, the correction factor is $0.85 + (0.946 \times 0.15) = 0.9919$. F_{ob} is given by:

$vF_{ob} = F/0.9919$…….. Equation 10

Adjustments for water absorbed by the skin (F_a): For non-oral water intake in the infant (F_a), a correction factor is necessary for environmental water influx to the baby, which is composed of atmospheric water absorbed through the skin and the lungs. Alveolar exchange is the largest component. Non-oral water intake is estimated as 6.3% of total water intake. As total water intake is equal to total water output, F_a is given by: $F_a = 0.063(F_{ob} + F_g)$

Calculation of oral water intake from sources other than human milk Fs: $F_s = F_{ob} + F_g - F_m - F_a$**.** There is an error associated with the estimate of the baby's intake of water from sources other than human milk, because of the assumptions made in this calculation. This error (25 ± 62 mL/d) results in a small apparent intake of water from sources other than human milk in babies who are truly exclusively breastfed.

3.6.4 Determination of body composition by deuterium oxide dilution method

To determine infants body composition, infants were given a fixed standardized dose of deuterium labelled water which had been accurately weighed as explained by International Atomic Energy Agency (IAEA) in Vienna, Austria (IAEA, 2009). A pre-dose sample of 2ml of saliva was taken from the infant's mouth by

using a passive cotton ball soaking collection method and marked as pre-dose (T0). Then 15 ml of deuterium oxide solution (2.5g deuterium and 12.5 ml of mineral water- equivalent to 0.5g/kg/body weight) was given to the infant orally via a syringe barrel taken. Post-dose saliva samples were taken at 2 hours and 3 hours. Infants were fasted for at least 30 minutes before the saliva samples were. All saliva samples were collected into a tightly capped cryogenic tube and kept in a freezer -20^{o}C awaiting transportation. Samples were then transferred in dry ice package to the Kenya Medical Research Institute (KEMRI) nutrition laboratories in Nairobi, Kenya. At this laboratory, enrichment of deuterium in saliva samples was determined using Fourier Transform Infrared (FTIR) spectrophotometer (Shimadzu, Vienna, Austria). Using the mean of deuterium enrichment based on the two post-dose samples, the dilution space and total body Water (TBW) were calculated as described by IAEA (IAEA, 2009). Fat free mass (lean mass) was calculated as: TBW/0.79 for both sexes. Fat Mass (FM) was computed by subtracting the FFM from the subject weight. Free Fat Mass Index (FFMI) and the Fat Mass Index (FMI) were computed by dividing individual infant's FFM and the FM by the squares of their respective height.

3.7 Data quality control

Before the commencement of the research, the project nurse and 5 enumerators were trained on research ethics and all data collection protocols (Annex 2 and 3). Before the beginning of the 6 months post-partum follow-up of mothers, re-fresher training was done. All tools were pre-tested before use. A questionnaire administered by an enumerator was reviewed by a different enumerator after every interview and corrections done before releasing the study respondents. All aspects were checked during this review and it was ensured that all questions were filled. At the end of each working day, the PhD student ensured that the saliva vial was accurately labeled and that the labeling matched the record in the questionnaires. In addition, the PhD student (resident in the field) and his supervisors (when in the field) checked if all ethical and data collection procedures were well adhered to. The field team held bi-weekly meetings to review progress and discuss solutions to challenges in data collection and entry.

3.8 Data handling and statistical analysis

3.8.1 Data entry and cleaning

Data was entered in EPI software and transferred in MS-Excel^{TM} for cleaning. The EPI templates for data entry were programmed to flag any entry that was out of the expected. For example, at recruitment, entries for age that indicated that the infant was more or less than 6 weeks old were flagged. Data from the used standard questionnaire was entered in the field 1-2 days after collection. In case a question was left out unfilled or the response was thought to be dubious, then the mother was called to confirm the figure. Z score values flagged by ENA for Smart 2011 were also not used for analysis. All statistical analyses were performed using the Statistical Package for Social Scientist version 20 (SPSS Vs 20).

3.8.2 Statistical analysis

3.8.2.1 General characteristics and breastfeeding practices

The Chi-squared test for 2 by 2 using Phi and Cramer's statistics (for categorical variables) and student's t-tests (for continuous variables) were used to detect differences between the two groups. Due to the differences in maternal age and education between the two groups of mothers (Table 4.1), these variables were controlled for in the multivariate analyses. Adjusted Odds Ratios were reported together with the p-values.

3.8.2.2 Breast milk intake

Differences between breast milk intake and other numerical variables between HIV-EU and HIV-U were compared using student's t-test statistics for independent variable (+SD). For categorical variables, Phi and Cramer's V statistics for 2 by 2 cross tabulations were used to detect difference. The p-values were generated at $\alpha=0.05$ and $p<0.05$ reflected the statistical significance. An infant who consumed <25g/day of non-human milk food per day was considered to have been exclusively breastfed within the 14 days of breast milk intake determination (Haisma et al., 2003). Infants at 6 weeks of age are expected to

consume 700g/day of breast milk (da Costa et al., 2010) while those who are 6 months of age are expected to consume 800g/day. For the respective ages, intakes below these amounts were considered as sub-normal. The odds of the 2 groups of infants taking below 700g/day of human milk/day (at 6weeks) and below 800g/day (at 6 months), was adjusted for maternal age, maternal years of education, food security, season, infant sex and infant birth weight and reported as aOR (adjusted odds ratio): (95% CI), p-values. In a multivariate analysis a regression model associated infant breast milk intake (and change in breast milk intake) as a dependent variable and maternal and infant variables as the independent variables while controlling for maternal HIV status, maternal education and maternal age. The yielded coefficient of correlations (r- coefficient) were reported together with the p-values at α=0.05.

3.8.2.1 Growth

Standard normal deviates (ZZ scores) were computed using the ENA for Smart 2011 using WHO Child Growth Standards (WHO Multicenter Growth Reference Study Group 2006). The WHO Global Database on Child Growth and Malnutrition uses a Z score cut-off point of <-2 SD to classify low weight-for-age (WAZ), low length-for-age (LAZ) and low weight-for-height (WHZ) as moderate and severe undernutrition, and <-3 SD to define severe undernutrition. Differences in Z scores for LAZ, WAZ and WAZ between two groups were compared using the students t-tests at α= 0.05 and p-values <0.05 reflected statistically significant difference. A linear regression between the growth and infant and maternal variables (shown in Figure 2.1) was run to identify potential confounders for which adjustment was necessary. Variables which yielded p<0.1 were controlled for in analyses. Some variables identified in literature were also included. For the linear regression analysis, all infants were grouped together as HIV-uninfected infants to identify some correlates of growth. Those with only one point measurement were excluded. One hundred and twenty three (126) cases were considered for this analysis that is those who were measured both at weeks 6 and 6 months of age points. For analysis involving breast milk intake, likewise only infants whose measurements were done both at week 6 and month 6 were

considered (n=121). The change in Z scores between 6 weeks and month 6 of the infant life were correlated with maternal and infant variables. Statistical significances were tested at both 0.01 and 0.05 levels.

The odds ratios for proportion of infants being <-2 Z scores among the two groups were adjusted for maternal height (due to the reported link- Cooper *et al.*, 2001; Varela-Silva *et.al.*, 2009). Infant sex was also adjusted for due to sex variations in growth as has been reported (Baig-Ansari *et al*., 2006; Wamani *et al.*, 2007). Maternal % fat mass, infant birth weight and breast milk intake were also adjusted due to the positive correlation with infant LAZ ($p<0.1$) at recruitment. Maternal age and education were also adjusted for due to the difference between the HIV-positive and negative mother in these variables.

3.8.2.1 Body composition

Differences in fat free mass, fat mass, % fat mass, fat free mass index and fat mass index between two groups were compared using the students t-tests at $\alpha= 0.05$ and p-values <0.05 reflected statistically significant difference. An initial regression between infant lean mass and infant and maternal variables was run to identify potential confounders. Maternal % fat mass, infant birth weight and breast milk intake were also adjusted due to the positive correlation with lean mass ($p<0.1$). Some variables were also identified in literature as potential confounders and adjusted for. They were maternal height (Cooper *et al*., 2001; Varela-Silva et.al., 2009). Infant sex was also adjusted for due to potential sex variations (Baig-Ansari *et al*., 2006; Wamani et. al, 2007). Maternal age and education were also included as covariates due to the differences between the HIV-positive and negative mothers.

3.9 Ethical Considerations

Ethical approval and annual renewals were secured from Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UON/ERC) (Annex 6.1). Only the mothers who consented were recruited and followed-up. All the identifier information were separated from other data.

All mothers and infants requiring medical care were referred for treatment and other services provided at the Siaya County Referral Hospital. No adverse effects were reported during the study. All HIV testing conducted as part of research were accompanied by appropriate counselling in line with the policies outlined in the Kenya guidelines for HIV testing (GOK, 2008).

CHAPTER FOUR

4 BREASTFEEDING BY HIV-INFECTED AND UNINFECTED KENYAN WOMEN AT 6 WEEKS AND 6 MONTHS POSTPARTUM

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4.1 Abstract

Background: Up-to-date breastfeeding recommendations for both HIV-positive and negative mothers are analogous, but there is paucity of data depicting how sound HIV-positive mothers adhere to the recommendations.

Objective: To compare the breastfeeding practices of HIV-positive and negative mothers of HIV-uninfected infants at 6 weeks and 6 months postpartum.

Methods: Mother-reported exclusive breastfeeding (EBF) were assessed cross-sectionally at 6 weeks and 6 months postpartum among 75 HIV-positive and 68 HIV-negative systematically selected from women attending postnatal care at Siaya County Referral Hospital, Kenya. The amount of non-human milk water intake by the infant was determined using dose-to-mother deuterium oxide dilution (DO) technique and used to validate self-reported EBF practices.

Findings: Mother-reported EBF rates at 6 weeks were 95.5% among the HIV-positive and the 75.8% among HIV-negative and rates were different between the two groups (p=0.003). EBF rates from the deuterium oxide dilution (DO) techniques were 23.3% and 14.5% for HIV-positive and HIV-negative mothers respectively (p=0.21). At 6 months postpartum, mother-reported rates were 75.0% among HIV-positive mothers and 59.7% among HIV negative mothers (p=0.071). There was a significant difference in DO determined EBF rates at 6 months

(43.3% and 24.2% for HIV-positive and HIV-negative mothers respectively; p=0.025).

Conclusions: Infants of HIV-positive mothers are more likely to be exclusively breastfed compared to infants of HIV-negative mothers. Validation of self-reported EBF practices with the low-cost, non-invasive deuterium oxide dilution technique is highly recommended to facilitate more effective breastfeeding promotion campaigns.

Key words: HIV-exposed, uninfected, exclusive breastfeeding, care practices

4.2 Introduction

Due to the risk of transmission of the virus through breast milk, HIV infection has over years presented a challenge on how HIV-positive mothers should breastfeed their infants. There have been shifts in breastfeeding in the context of HIV recommendations over time. In 1997, the global recommendation to health workers was to encourage breastfeeding and only advise HIV-infected mothers against breastfeeding if infectious diseases were not a major cause of death during infancy in their respective regions (Vinther and Helsing, 1997). At that point, there was room given for the HIV-positive mother not to breastfeed in the effort to balance a baby's risk of becoming infected with HIV through breastfeeding against its risk of dying of other causes if not breastfed. This was followed by the recommendation to avoid all breastfeeding by HIV-infected mothers when replacement feeding was acceptable, feasible, affordable, sustainable and safe (WHO, 2003). Avoidance or early cessation of breastfeeding seemed logical or appropriate at that time (UNICEF, 2015). However, the current guidelines on HIV and infant feeding recommend that irrespective of maternal HIV status and that of infants, mothers should exclusively breastfeed up to 6 months of the age of infants (WHO, 2010). There is however, paucity of data on how the current breastfeeding recommendation impacts breastfeeding practices of HIV-positive mothers. The EBF is even more important for infants who despite being HIV-exposed in-utero or via breastfeeding are HIV-negative. EBF rate is an imperative variable measured in infant and young child feeding/nutrition surveys,

and globally the country-specific DHS collects the data every five years. Traditionally, EBF rates have been based on maternal recall of what was fed to an infant 24 hours preceding the interview. The recalls are largely cross-sectional and liable to recall errors as well as social desirably bias because mothers may report practices they have been told are good. It is therefore reasonable that an objective indication of EBF is an important programmatic data. Dose-to-mother deuterium oxide dilution (DO) technique is a stable isotope technique applicable to objectively assess whether a mother is exclusively breastfeeding. The DO technique assesses breast milk and non-human milk water intake data over a 14-day period and is useful for validation of mother-reported breastfeeding practices based on recall. The study aimed at assessing and comparing EBF practice between HIV-positive mothers whose infants are not HIV-infected and that of HIV-negative mothers.

4.3 Methods

4.3.1 Study design and setting

This was part of a study designed to compare breast milk intake between HIV-uninfected infants whose mothers are HIV-1 infected and infants whose mothers are HIV-1 negative. Mothers recalled breastfeeding practices and breast milk intake was assessed using stable isotope technique (dose-to-mother deuterium oxide dilution technique) which also estimates the non-human milk water intake. The study was based at the Maternal and Child Health (MCH) Clinic of Siaya County Referral Hospital in Western Kenya. Mothers were approached to enrol into the study as they brought their infants for post-natal care between February 2014 and September 2014. Siaya County is a resource-poor area in the Lake Victoria Region of Kenya where HIV rates are one of the highest in Kenya (15.9% and 23.3% among men and women, respectively) (NAAC, 2015).

4.3.1 Sample size

The sample size was computed based on the objective of comparing breast milk intake of HIV-uninfected infant born of HIV-1 positive and negative mothers, as explained by Kirkwood and Sterne (2003) as shown in the equation 1.

$$\frac{(u+v)^2(\delta_1^2+\delta_0^2)}{(\mu_1-\mu_0)^2} \quad \text{.....(Equation 1)}$$

Where
u= One sided % point of normal distribution corresponding to 100%- the power=1.52;
v=% point of the normal distribution corresponding to two sided significant level=1.28;
$(\mu_1 - \mu_0)$= Expected difference in means; and
δ1, δ0= Standard deviations (s.d).

A mean difference of 10g/kg body wt/day in breast milk intake between HIV-EU and HIV-U infants and Standard Deviation (SD) of 18 g/kg body wt/day was adopted (Galpin *et al.,* 2007). With a statistical power of 80% and 5% level of significance, a minimum sample size of 50 mother-infants dyads for each of the two age cohorts was required. With an attrition rate of 20%, a minimum sample size of 60 for each group was arrived at.

4.3.2 Recruitment of mothers

Seventy five HIV-positive and 68 HIV-negative mothers attending post-natal clinic for first set of child vaccinations at 6 weeks postpartum and or first maternal gynaecological check-up were systematically sampled, recruited into the study and followed up at 6 months of age. Based on MCH attendance register, it was estimated that ~10-20 mothers would attend clinic daily. The MCH was operational from Monday to Friday every week. To achieve the desired sample size, the study aimed to recruit ~5-10 mothers-infant dyads per day. Therefore we approached every second mother in the queue at the clinic.

4.3.3 Inclusion and exclusion criteria

Mother-infants dyads of 6 weeks old HIV-uninfected infants were eligible for this study. The following infants were excluded: infants having <2500g birth weight, preterm infants, infants not able to breastfeed, infants or mothers showing signs of

chronic illnesses (as was determined by the project nurse). To minimise loss to follow-up, those intending to move away from Siaya District 7-10 months from the date of recruitment into the study were also excluded.

4.3.4 Maternal and infant HIV testing

Maternal HIV status at 6 weeks partum and 6 months after birth was determined by antibody testing by Colloidal Gold, (KHB Shanghai Kehua Bioengineering Co. Ltd). At 6 weeks of age, infants were tested with HIV-1 DNA polymerase chain reaction (PCR) using T100 Thermal Cycler (Bio-Rad Laboratories Inc, UK). It was not possible to test the HIV-EU infants at 6 months of age because of the Kenya government guidelines on HIV infants which stipulates that the test should be done when the infants are 9 months old using an antibody test and confirmed with PCR (GOK, 2012). The HIV-EU infant's HIV tests were thus done by 9 months to confirm if the infants were still HIV-negative. Two HIV-EU infants seroconverted at 9 months and were thus excluded from the study.

4.3.1 Data collected

Using a structured questionnaire, data on socio-demographic and economic status, timely initiation of breastfeeding, exclusive breastfeeding, expression of breast milk, counselling on breastfeeding were collected by recall at recruitment (6 weeks postpartum) and at follow-up (6 months of age of infants). WHO definitions of breastfeeding practices were adopted (WHO, 2008). Timely initiation of breastfeeding was defined as putting infants to breast within one hour of birth. Exclusive breastfeeding was defined as feeding infants solely on breast 24-hours preceding the interview. Standard DHS question of recalling 24 hour infant intakes of the following foods was adopted: breast milk, plain water, sugar or glucose water, gripe water, sugar-salt-water solution, fruit juice, infant formula, tea infusion, honey, milk, porridge and honey.

4.3.2 Determination of exclusive breastfeeding using DO technique

Breast milk intake was measured at 6 weeks and 6 months of age of infant using the dose-to-mother DO technique as explained by IAEA (2010). After being

weighed, baseline (pre-dose) saliva samples were collected from the mother and the infant (day 0- T_o) using a sterile cotton wool and syringe. Mother were then be given 30g of deuterium oxide ($^2H_2$ O) through a straw. They were then instructed to feed the infant as usual and allowed to go home. At least 2ml of saliva samples were subsequently be collected from mother and infant in 10ml polypropylene sterile tubes on days 1, 2, 3, 4, 13 and 14 (corresponding toT_1, T_2, T_3, T_4, T_{13} and-T_{14}). It was ensured that post-dose saliva collection is done at the same time as time for baseline saliva collection on day 0 (T_o). All labelled tubes polypropylene sterile tubes were separately secured in zip-lock polythene bags and immediately frozen in -20oC freezers in Siaya County Referral Hospital. Samples were then transported in cool boxes for to Kenya Medical Research Institute (KEMRI) nutrition laboratory in Nairobi, Kenya. Deuterium enrichment in mother/infant saliva over a 14-day period was measured against a standard in the Fourier Transform Infrared Spectrophotometer (FTIR 8400 Series, Shimadzu Corporation, Kyoto, Japan) at KEMRI nutrition laboratory. Human milk intake and intake of water from other sources other than breast milk was then calculated using a spread sheet developed by IAEA (IAEA, 2010). This method estimates also the non-human milk intake (indicative of consumption of liquids/foods other than breast milk) in grams/day. An infant who consumed <25g/day of non-human milk food per day was considered to have been exclusively breastfed within the 14 days of mother and infant saliva collection days (Haisma *et al*., 2003).

4.3.3 Data entry and analysis

Data was entered in EPI6 and cleaned using MS-ExcelTM before transferring to Statistical Package for Social Scientists (SPSS) version 20 for analysis. Some mothers-infant dyads were lost to follow-up. The Chi-squared test for 2 by 2 (for categorical variables) using Phi and Cramer's statistics (for categorical variables) and student's t-tests for independent samples (for continuous variables) were used to detect differences between the two groups. Due to the differences in maternal age and education between the two groups of mothers (Table 4.1), these variables were controlled for in the multivariate analyses. Adjusted Odds Ratios were reported together with the p-values.

4.3.4 Ethical considerations

Ethical approval was secured from Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UON/ERC). Only the mothers who gave written consent were enrolled and followed up.

4.4 Results

4.4.1 General characteristics of study mother-infant dyads

Table 4.1 shows the socio-demographic and economic characteristics of HIV-positive and HIV-negative mothers at enrolment (6 weeks postpartum). HIV-infected mothers were older (p=0.002) and less educated than the uninfected mothers (p=0.035). Majority of the mothers were multigravidea with male infants. The sex composition of the infants of HIV-positive and negative mothers was comparable.

Table 4.1: Socio-demographic characteristics of HIV- positive and HIV-negative mothers at enrolment (6 weeks postpartum)

Characteristics	HIV-1 positive mothers (n=75)	HIV-1 negative (n=68)	P-value∞
Mean age of the mother ±SD	28.8±5.9	25.6±6.4	0.002
Primigravidae, % (n)	13.3 (10)	17.6 (12)	0.47
Infant sex	61.3	45.6	0.059
Attended secondary school and above, % (n)	16.0 (12)	30.9 (21)	0.035
Living in the villages (% rural), % (n)	69.3 (52)	61.8 (42)	0.34
% living in grass thatched houses	52.2 (24)	62.6 (22)	0.96
Marital status, % (n)			
Single	6.9 (5)	17.6 (12)	.0.058
Married	87.5 (63)	77.9 (53)	
Divorced	1.4 (1)	4.4 (3)	
Living with spouse/partner, % (n)	72.0 (54)	70.6 (48)	0.34
Polygamous marriages[¥], % (n)	12.9 (9)	9.4 (6)	0.55
Religion, % (n)			
Catholic	25.3 (19)	17.9 (12)	0.324
Protestants	74.7 (56)	80.9 (55)	
Place of delivery, % (n)			
Home	13.3 (11)	13.2 (9)	0.804
Clinic	6.7 (5)	4.4 (4)	
Hospital	78.7 (56)	82.4 (56)	
Caesareans deliveries, % (n)	8.0 (6)	7.4 (5)	0.88
On ART [Ω], % (n)	60% (43)	-	-

[∞]Comparing the HIV-1 positive and HIV-1 negative mothers. For numerical variables, students t-test for independent samples at α= 0.05. For categorical variables, Phi and Cramer's V statistics at α= 0.05 used

[¥]For only those who are married

[Ω] Only among HIV-positive mothers

About two-thirds of the study participants were dwelling in the villages the rest in towns. Most women were married, in monogamous arrangements, living together with their partners and were Christians. Most mothers delivered in health facilities (hospital and clinics) and had vaginal deliveries for the infants under study. The economic status of the two groups was also comparable as shown by the types of housing (p=0.96).

4.4.2 Infant feeding practices at 6 week and month 6 after birth

Table 4.2 depicts the breastfeeding practices among mothers at 6 weeks and 6 months postpartum. More than one half of the mothers in both groups initiated breastfeeding timely (within the first hour after birth) and this was close to the Kenyan national average of 61.3% (GOK, 2008). Only a small proportion of the mothers expressed breast milk both at 6 weeks postpartum and 6 months of age and this confirmed the anecdotal evidence that this practice is uncommon in the study community. Most mothers in both groups had been counselled on breastfeeding during the perinatal period and after 6 weeks.

Infants who consumed >25g/day of non-human milk water as indicated by DO technique were considered not to be EBF (Table 4.3). Disproportionately lower figures of EBF were found using DO technique compared to maternal recall both at 6 weeks postpartum at 6 months of age. At 6 weeks postpartum, a greater proportion of HIV-positive mother exclusively breastfeed than the negative counterparts (p=0.003) as determined by mother recall. EBF rates from the DO techniques was not significantly different among the two groups (23.3% and 14.5% for HIV-positive and HIV-negative mothers, respectively; p=0.21). Therefore at 6 weeks, EBF rates based on recall were 4 and 5 times higher than the EBF rates based DO for HIV-positive and HIV-negative mothers, respectively. There was no significant difference in mother-reported rates of EBF at 6 months (75.0% and 43.3% for HIV-positive and HIV-negative mothers, respectively; p=0.071).

Table 4.2: Feeding practices and counselling from birth to 6 months postpartum

Practices	Proportion of mothers			
	HIV +ve	HIV-ve	aOR (95% CI) [∞]	P-values
From birth to 6 weeks postpartum	**(n=75)**	**(n=68)**		
Initiated breastfeeding within an hour from birth	52.0	57.4	1.2 (0.6-2.4)	0.51
% expressing breast milk	2.7	1.5	0.4 (0.03-4.8)	0.47
% counselling on breastfeeding at perinatally	82.7	72.1	1.22 (0.49-3.0)	0.14
6 months of age of infants	**n=63**	**n=63**		
% expressing breast milk	3.2	1.5	0.32 (0.02-4.1)	0.38
% counselling on breastfeeding between week 6 and months 6 after birth	79.0	80.6	1.2 (0.5-2.8)	0.75

[∞]Adjusted Odds Ratio (aOR) at 95% Confidence Interval (CI) at 95%. Adjusted for the maternal education and maternal age

[¥]Breast pains in one or both breast since birth to 6 months after birth

The DO technique determined rates at 6 months postpartum were 43.3% and 24.2% for HIV-positive and HIV-negative mothers, respectively (p=0.025). Therefore at 6 weeks, the EBF rates based on recall were 1.7 and 2.5 times higher than the EBF rates based on DO technique for HIV-positive and HIV-negative mothers, respectively.

Table 4.3: Exclusive breastfeeding as determined by isotopic technique and as maternally recalled

Variable	Exclusive breastfeeding rate (%)		P-value
6 weeks	**HIV-EU (n=60)**	**HIV-U (n=62)**	
Deuterium oxide determined	23.3	14.5	0.21
Maternal recall	95.5	75.8	0.003
6 months			
Deuterium oxide determined	43.3	24.2	0.025
Maternal recall	75.0	59.7	0.071

4.5 Discussion

In this study, we compared the EBF practice between HIV-positive and negative mothers using recall method and validated this with the DO technique. HIV-positive and negative mothers were comparable in demographic and socio-economic characteristics except for their ages and education status. A greater proportion of HIV-positive mothers than the HIV-negative practiced EBF. Maternal recall of exclusive breastfeeding overestimated the EBF rate. The results of this study were however only limited to the mother who attend the post-natal clinic and not for the general population.

The EBF rates determined by DO technique are far more depressed as compared to the maternally recalled EBF rates and it is apparent that mothers over-report EBF. This is consistent with a Cameroonian study (Medoua *et al*., 2012). Although good accuracy in reporting of feeding practices by Bangladeshi mothers was comparable to the objective DO technique, apparent mis-reporting was widely present (Moore, 2007). There is a possibility in the present study that majority of mothers report what they are expected to practice (as informed by health workers) rather than the actual practice. The observation that the HIV-positive mothers exclusively breastfed more could be explained by the varying level exposures to counselling and messages (on infant feeding in the context of HIV) among the two groups of mothers. Kenya fully subscribes to the WHO recommendations on infant feeding in the context of HIV (GOK, 2013). In this

present study by 6 weeks postpartum, 10% more of the HIV-positive mothers than HIV-negative had been counselled on breastfeeding. In addition to the interaction with the health workers, most HIV-positive mothers are members of HIV-positive mother support groups where mothers share experiences and psychosocially support each other to care for their infants. In these forums, more health messages are passed on. The HIV-positive mothers are thus more exposed to health and nutrition messages than their HIV-negative counterparts. In earlier findings (before WHO 2010 guidelines of infant feedings), exclusive breastfeeding rates were comparatively lower among the HIV-positive mothers and formula feeding and exclusive formula feeding were more acceptable among this group of mothers (Bland *et al*., 2008; Rollin *et al*., 2004). About a decade prior the advent of the current breastfeeding recommendations, a Kenyan study in a resource poor setting found (by maternal recall) that at 6 weeks postpartum, only 30% of HIV-EU exclusively breastfed and by 4.5 months, none were being exclusively breastfed (Sherry *et al*., 2000). One reason for such observations was that the HIV-positive mothers were not able to afford breast milk replacements (alternative feeds) (Kiarie *et al*., 2004). It is apparent from this present study that the tendency of the HIV-positive mothers not to breastfeed exclusively as compared to the HIV-negative mothers has been overturned, and now we even observe the HIV-positive practicing EBF more than the negative counterparts. The infant HIV sero-status is also an important factor and underscores the effect of Prevention of Mother-to-Child Transmission (PMTCT) programs. The knowledge (among the HIV-positive mothers) that their infants are uninfected could be a motivation to adhere to the WHO 2010 guidelines in order to further protect the infants from the virus transmission through breast milk and to raise health children. The provision of combination ART (Tenofovir- Effavirenz combination or Tenofovir- Effavirenz-nevirapine combination) and antibiotics (septrin) to HIV-positive mothers also ensures they are less affected by opportunistic illnesses which may usually affect their ability to effectively care for and breastfeed their infants. Sixty-percent of HIV-positive mothers in this study were on ART.

4.6 Conclusions and Recommendations

Infants of HIV-positive mothers are more likely to be exclusively breastfed compared to infants of HIV-negative mothers. Validation of self-reported EBF practices with the low-cost, non-invasive deuterium oxide dilution technique is highly recommended to facilitate more effective breastfeeding promotion campaigns.

4.7 Acknowledgements

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4.8 Funding

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4.9 Competing Interests

The authors declare that they had no competing interest before and during the research.

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CHAPTER FIVE

5 EFFECT OF MATERNAL HIV STATUS ON BREAST MILK INTAKE OF HIV-UNINFECTED KENYAN INFANTS AT 6 WEEKS AND 6 MONTHS POSTPARTUM

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5.1 Abstract

Background: It is currently recommended that all infants irrespective of the HIV status of their mothers be exclusively breastfed for the first 6 months of their lives. It is however not know if breast milk intake of infants born of HIV-positive mothers is different from that of those born of HIV-negative mothers.

Objective: To compare the breast milk intake of HIV-exposed, uninfected (HIV-EU) infants and that of HIV-unexposed (HIV-U) infants.

Method: Breast milk intake was measured from 60 HIV-uninfected infants born of HIV-positive and 61 born of HIV-negative mothers using isotopic technique (deuterium oxide dose-to-mother technique) at 6 weeks postpartum and 6 months of age. Pre-dose saliva samples were obtained from both the infants and the mothers on day 0. Mothers then received a 30g dose of deuterium oxide. Post-dose saliva samples were collected from both infants and mothers over 14 days on days 1, 2, 3, 4, 13 and 14 and then analyzed for deuterium enrichment. Data on socio-demographic and economic characteristics, maternal and infant anthropometries were also collected.

Results: At 6 weeks postpartum HIV-exposed uninfected infants (HIV-EU) consumed a mean of 717g/day of breast milk and this was comparable to 712.6g/day consumed by HIV-unexposed (HIV-U) (p=0.86). The 131.4g/day of non-human milk water consumed by HIV-U at 6 weeks was significantly higher than 78.1g/day consumed by HIV-EU (p=0.024). At 6 months after birth HIV-EU consumed 960.8g/day while HIV-U consumed 963.1g/day (p=0.95) and these

amounts were comparable. At 6 months of age infants' consumption of non-human milk water was comparable (p=0.13). Among the HIV-positive mothers, those on antiretroviral (ART) and those not ART did not have different breast milk output (p=0.28 at week 6 and 0.91 at month 6). Mothers with CD4≤350 and CD4>350 cells/m-3 also had comparable breast milk output (p=0.32 and 0.99 at week 6 and month 6 respectively). Factors positively associated with elevated breast milk intake among HIV-uninfected infants were maternal BMI (r=0.247 at 6 weeks), maternal lean mass (r=0.270 at 6 weeks and 0.365 at 6 months), infant birth weight (r=0.345 at 6 weeks) and infant current weight (r=0.486 at 6 weeks and 0.557 at 6 months).

Conclusion: Exposure to HIV does not affect the amount of breast milk a Kenyan HIV-uninfected infant 6 months old and below consumes. Interventions that ensure normal maternal body mass index, maternal lean mass, infant size (birth and current weight) are important in increasing breast milk intake of HIV-uninfected infants.

5.2 Introduction

Currently, the health and nutrition status of the HIV-exposed, uninfected (HIV-EU) infants present a public health concern. Due to widespread of programs on Prevention of Mother To-Child Transmission (PMTCT) of HIV, their numbers are on the increase. Freedom from HIV does not however guarantee optimal health, nutrition and development of infants born of HIV-positive mothers. HIV-positive mothers have poor pregnancy outcomes (Rollins *et al.,* 2007) and their infants who are mostly HIV-uninfected have hematological and immunological challenges (Kilted *et al.,* 2011; Reikie *et al.,* 2014). In resource poor settings, HIV-EU experience heightened morbidity, mortality and limited growth early in their lives. Out of the key potential factors causing their poor health and nutrition, one that is most modifiable is infant feeding practices (Filteau, 2009). In resource poor settings breastfeeding is currently the most important infant feeding practice due to the lessened propensity to afford breast milk substitutes. Currently, the WHO recommendation for all infants is to be exclusively breastfed for the first

half of infancy irrespective of the maternal and infant HIV status. However, infants born of and cared for by HIV-positive mothers may have altered amount of breast milk intake. Studies have shown higher levels of subclinical mastitis in HIV-infected women (Kasonka *et al.,* 2006) and reduced breast milk output among dairy cows with subclinical mastitis has also been reported (Shuster et. al, 1995). Using latest techniques such as isotopic techniques, the infants' intakes have been determined in various parts of the world for different age groups and set-ups (Haisma *et al.,* 2003; da Costa, *et al.,* 2010). However, the amounts consumed by infants born of HIV-positive mothers still remain a research gap. This study thus aimed at assessing the breast milk intake of HIV-uninfected infants born of HIV-positive mothers as compared to intake of those born of HIV-negative mothers at 6 weeks postpartum and 6 months after birth. The study also aimed at highlighting on the factors that are associated (drivers) of breast milk intake among HIV-uninfected infants.

5.3 Subjects and Methods

5.3.1 Study design and population

This was prospective cohort study designed to measure breast milk intake of HIV-uninfected infants born of HIV-positive (study group) and HIV-negative mothers (control) at 6 weeks post-partum and at 6 months after birth using stable isotope technique (deuterium oxide dose-to-mother technique). The study was set in a Maternal and Child Health (MCH) Clinic of Siaya County Referral Hospital in Western Kenya. Mother-infant dyads were recruited into the study as they attended post-natal care between February 2014 and September 2014. Siaya County is in the Lake Region of Kenya where HIV rates are highest. Siaya County is inhabited by predominantly rural population with a HIV prevalence of 15.9% among adult men and 23.3% among adult women. These rates are way above the national average of 5.6% and 7.6% among adult men and women respectively (NAAC, 2015).

The sample size was computed based on the aim of comparing breast milk intake of HIV-uninfected infant born of HIV-1 positive and negative mothers using a statistical approach by Kirkwood and Sterne (2003) as shown in the equation 1.

$$\frac{(u+v)^2(\delta_1^2+\delta_0^2)}{(\mu_1-\mu_0)^2} \quad \text{..... (Equation 1)}$$

Where
u= One sided % point of normal distribution corresponding to 100%- the power=1,52;
v=% point of the normal distribution corresponding to two sided significant level=1.28;
$(\mu_1 - \mu_0)$= Expected difference in means; and
δ1, δ0= Standard deviations (s.d).

A mean difference of 10g/kg body wt/day in breast milk intake between HIV-EU and HIV-U infants and Standard Deviation (SD) of 18 g/kg body wt/day was adopted (Galpin *et al.,* 2007). With a statistical power of 80% and 5% level of significance, a minimum sample size of 50 mother-infants dyads for each of the two age cohorts was required. With an attrition rate of 20%, a minimum sample size of 60 for each group was arrived at. Only infants whose breast milk data was collected at both 6 months and 6 weeks were used for the analysis. These were 60 HIV-positive mothers/HIV-EU infant dyads and 61 HIV-negative mothers/HIV-U infant dyads as shown in the study profile in Figure 3.1 in chapter 3 of this manuscript.

5.3.2 Mother-infant dyads sampling

Mothers coming for first postnatal clinic for gynaecological check-up and/or for infant vaccinations were systematically sampled. Using the hospital registers, it was estimated that on a busy day of the week, about 20 mother-infant dyads were expected at the postnatal clinic. The target was to consent half of post-natal attendance each day. Alternate mothers were considered for the study. The first mother to line up for the antenatal care was consented and second was not considered unless the preceding mother declined to participate in the study. The process was repeated until the end of each working day at the MCH.

5.3.3 Inclusion and exclusion criteria

Mother-infants dyads of 6 weeks old HIV-uninfected infants were eligible for this study. The following infants were excluded: infants having <2500g birth weight, preterm infants, infants not able to breastfeed, infants or mothers showing signs of chronic illnesses (as was determined by the project nurse). To minimise loss to follow-up, those intending to move away from Siaya District 7-10 months from the date of recruitment into the study were also excluded.

5.3.4 Mother-infant dyads sampling

The following mothers-infant dyads were excluded from the study: those with infants having <2500g birth weight, with preterm infants, those not able to breastfeed and mothers or infants showing signs of chronic illnesses. To minimise loss to follow-up, those who intended to move far away from their then current locales 6 months from the time of recruitment were also excluded.

5.3.5 Maternal and infant HIV testing

Maternal HIV antibody tests were done using a diagnostic Kit for HIV (1+2) Antibody (Colloidal Gold) manufactured by KHB Shanghai Kehua Bioengineering Co. Ltd. Infants were tested with HIV-1 DNA polymerase chain reaction (PCR) at 6 weeks. At 6 months infants HIV test was done using antibody test and confirmed with PCR in accordance to the Government of Kenya guidelines (GOK, 2012). At recruitment, maternal blood samples were collected for routine CD4 cell count using the CD4 cell counter at Kenya Medical Research Institute Kisian laboratory, Kisumu, Kenya. It was not possible to test the HIV-EU infants at 6 months of age because of the Kenya government guidelines on HIV infants which stipulates that the test should be done when the infants are 9 months old using an antibody test and confirmed with PCR (GOK, 2012). The HIV-EU infant's tests were thus done at 9 months to confirm if the infants were still HIV-negative. Two HIV-EU infants seroconverted at 9 months and were thus excluded from the study. It could not be determined the exact time of sero-conversion.

5.3.6 Saliva samples collection and analysis

Saliva samples were collected from the mother and the infant for a period of two weeks as explained in section 3.6.3 of this manuscript. Intake of human milk and water from sources other than human milk were then calculated by fitting the deuterium enrichment data to a model for water turnover in the mother and in the baby as explained in section 3.6.3 of this manuscript.

5.3.7 Anthropometry data collection

Mother height and infant length were measured at 6 weeks post-partum and 6 months after birth using the Infant/Child/Adult ShorrBorad (79" x 1/8" / 200 x 0.1 cm) measured to the nearest 0.1 cm. Maternal weight was determined by using Camry weighing scale (Model EB 9318), and recorded to the nearest 0.1 kg, while the nude weight of infants was measured using the Salter Electronic Baby (maximum 25kg). Shorr Tape (65 cm x 0.1 cm) and Shorr Child MUAC Tape (26 cm x 0.1 cm) was used for determining the maternal and infant MUAC respectively. Infants head circumference was measured using the ShorrTape (65 cm x 0.1 cm). Triceps and subscapular skinfold-thickness measurements were done using Holtain skinfold calipers (Crymych, United Kingdom) to the nearest 0.1mm.

5.3.8 Other data sets collected

Maternal socio-demographic and economic data were collected at recruitment (6 weeks postpartum) using a structured coded questionnaire. The gestational age of the infants, date of birth and birth weight were obtained from the mother and child clinic cards, and confirmed by mother recall. *Siaya County Referral Hospital* based the determination of the gestational age on mother's last reported menstrual period. Birth weight was extracted from the mother and child health cards. Infant and maternal infections in the two weeks preceding the assessment was recalled by the mother at 6 weeks post-partum and 6 months of age of infants. Mothers were also asked if they had sufficient food in the last 4 weeks to indicate the food

security situation. The prevailing season was recorded as pre-harvest or harvest season.

5.3.9 Statistical analysis

Data was entered in EPI software and transferred in MS-Excel^{TM} for cleaning. Only mother-infants dyads with breast milk intake data at both 6 weeks and 6 months were included. All statistical analyses were performed using the Statistical Package for Social Scientist version 20 (SPSS Vs 20). Differences between breast milk intake and other numerical variables between HIV-EU and HIV-U were compared using student's t-test statistics for independent variable ($\pm$SD). For categorical variables, Phi and Cramer's V statistics for 2 by 2 cross tabulations were used to detect difference. The p-values were generated at α=0.05 and $p<0.05$ reflected the statistical significance. An infant who consumed <25g/day of non-human milk food per day was considered to have been exclusively breastfed within the 14 days of breast milk intake determination (Haisma *et al.*, 2003). Infants at 6 weeks of age are expected to consume 700g/day of breast milk (da Costa *et al.*, 2010) while those who are 6 months of age are expected to consume 800g/day. For the respective ages, intakes below these amounts were considered as sub-normal. The odds of the 2 groups of infants taking below 700g/day of human milk/day (at 6weeks) and below 800g/day (at 6 months), was adjusted for maternal age, maternal years of education, food security, season, infant sex and infant birth weight and reported as aOR (adjusted odds ratio): (95% CI), p-values. In a multivariate analysis a regression model associated infant breast milk intake (and change in breast milk intake) as a dependent variable and maternal and infant variables as the independent variables while controlling for maternal HIV status, maternal education and maternal age. The yielded coefficient of correlations (r-coefficient) were reported together with the p-values at α=0.05.

5.3.10 Ethical consideration

Ethical approval was secured from Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UON/ERC). Only the mothers who consented were recruited and followed-up.

5.4 Results

5.4.1 General characteristics

Mother-infant dyads for this study were drawn from the Maternal and Child Health clinic (MCH). Table 5.1 depicts the maternal general characteristics at recruitment (6 weeks postpartum). The HIV-positive mothers were older (p=0.003) and less educated (p=0.04) than the HIV-negative mothers. Majority of the two groups of mothers were married and were comparable (p=0.06). Mother-infants dyads with pre-terms were excluded from the study and the gestational ages between the two groups were normal and comparable (p=0.94). All mothers recruited were Christian, majority delivered the study infants at the hospital/clinic and most had normal/vaginal deliveries.

The mother-infant dyads from the two groups came from households with comparable economic status as indicated by the type of the house and the maternal income status (Table 5.1). The household food security and the season in which the two groups of mothers were recruited were different. A greater proportion of HIV-positive mothers reported they were food insecure than the HIV-negative counterparts.

Table 5.1: Maternal general characteristics at recruitment (6 weeks post-partum)

Characteristics	Value or proportion		
	HIV-positive (n=60)	HIV-negative (n=62)	P-value[∞]
Mean age of the mothers (years ±SD)	29.0 ±6.3	25.6 ±6.3	0.003
Birth status – Primigravidae (%)	15.0	17.7	0.68
Gestational age (weeks ±SD)	39.9 ±1.8	39.8±2.1	0.94
% delivered at the hospital/clinic	86.7	85.5	0.85
Caesareans delivery (%)	8.3	8.1	0.96
Mean number of own children <5 years old (number ±SD)	1.7 ±0.7	1.8 ±0.7	0.21
Proportion attended secondary school and above	15.0	30.6	0.04
Mean years in school	8.4 ±2.2	9.1 ±2.3	0.054
% married	93.3	82.3	0.063
Religion (%)			
Catholic	23.3	17.7	0.44
Protestants	76.7	82.3	0.44
% living in the villages (% rural)	65.0	64.5	0.96
% living in grass-thatched houses	31.7	33.9	0.80
% employed (earning income)	3.3	9.7	0.16
% household food insecure	76.7	50.0	0.008
% in pre-harvest season	65.0	95.2	0.000

[∞]For continuous variables, students t-test for independent samples at $\alpha = 0.05$, For categorical variables, Phi and Cramer's V statistics at $\alpha = 0.05$.

Among the HIV-positive mothers, ~two-thirds were on ART and about 32.6% had CD4 cell count <350cells/mm^3 (advanced and severe immunological status as defined by WHO, (2007). Most of the mothers were not ill in two weeks preceding the interview (Table 5.2) and this was comparable between the two groups (p=0.16).

Table 5.2: Maternal ART, CD4 cell and illness status at recruitment

Variable	HIV-positive (n=60)	HIV-negative (n=62)	P-value[∞]
% on ART	61.4	-	-
Mean CD4 cell count (cells/mm^3)[¥]	493.3 ±283.4	-	-
Immunological classification based on CD4 cell count (cells/mm^3)[¥]			
% with >500 (none or not significant)	30.7	-	-
% with 350-499 (Mild)	30.4	-	-
% with 200-349 (Advanced)	17.4	-	-
% with <200 (Severe)	15.2	-	-
% ill in the past 2 weeks	0.0	3.2	0.16

[∞]For continuous variables, students t-test for independent samples at $\alpha = 0.05$, For categorical variables, Phi and Cramer's V statistics at $\alpha = 0.05$.
¥Only for HIV-positive mothers. N=60 for ART and n=46 for CD4 count analysis.

The HIV-positive and negative mothers were also comparable in height, BMI and body composition ($p>0.05$) as shown in Table 5.3. The study only recruited infants who had >2,500g birth weight and those born of HIV-positive and negative mothers had comparable weights at birth (Table 4.3).

Infants of HIV-positive mothers had less length-for-age Z scores (LAZ) ($p=0.021$) and more weight-for-length Z scores (WAZ) ($p=0.046$) as compared to infants born of HIV-negative infants. The WAZ, weights, lengths and birth order of the two groups of infants were comparable.

Table 5.3: Maternal and infant anthropometry at recruitment (6 weeks postpartum)

Characteristics	**6 weeks post-partum**		
Maternal anthropometry and body composition[Ω]	**HIV-positive (n=60)**	**HIV-negative (n=62)**	**p-value[∞]**
Height (m ±SD)	160.3 ±6.1	160.7 ±6.2	0.74
BMI (kgm-2 ±SD)	22.5 ±3.4	22.7 ±3.3	0.73
% BMI <18.5cm	3.3	8.1	0.26
Fat-Free Mass (kg ±SD)	45.0 ±2.2	45.0 ±2.6	0.83
Fat mass (kg ±SD)	12.9 ±8.5	13.5 ±8.18.1	0.68
% Fat mass	20.7 ±9.9	21.8 ±10.1	0.53
Infant anthropometry	**HIV-EU (n=60)**	**HIV-U (n=61)**	**p-value[∞]**
Birth order ±SD	3.4 ±1.8	3.0 ±1.8	0.12
Birth weight in (kg ±SD) [¥]	3.2 ±0.43	3.3 ±0.47	0.15
Length (cm ±SD)	53.7 ±2.1	54.4 ±3.0	0.10
Weight (Kg ±SD)	4.7 ±0.6	4.8 ±0.7	0.46
LAZ ±SD	-1.1 ±1.0	-0.6 ±1.3	0.021
WAZ ±SD	-2.3 ±1.0	-0.05 ±1.0	0.212
WLZ ±SD	1.1 ±1.1	0.7 ±1.2	0.046

[∞]For continuous variables, students t-test for independent samples at α= 0.05, For categorical variables, Phi and Cramer's V statistics at α= 0.05

[¥]Data extracted from the mother and child health cards.

[Ω]Body composition as estimated from the maternal Total Body Water (determined by deuterium oxide technique)

5.4.2 Breast milk intake of infants at 6 weeks postpartum and 6 months of age

Table 5.4 depicts the amount breast milk and non-human milk water taken by the infants at 6 weeks postpartum and 6 months of age. At 6 weeks postpartum, absolute breast milk intake per day by HIV-EU (717g/day) and by HIV-U (712.6g/day) were comparable (p=0.86), as well as the amounts consumed per day/weight of the infant (p=0.63). Absolute amounts of breast milk consumed at 6 months by HIV-EU (960.8g/d) and HIV-U (963g/day) were comparable (p=0.91). Breast milk consumed per day/weight of infant were also comparable between the two groups (p=0.80). Lowest amount of breast milk consumed by the infants was 430g/day and 706g/day at 6 weeks and 6 months after birth respectively. Highest amounts consumed were 1321g/day and 1270g/day at 6 weeks and 6 months after birth respectively. Infants born of HIV-negative mothers consumed a greater amount of non-human milk water compared to those born of HIV-negative

mothers (p=0.024). This depicted higher consumption of other fluids other than breast milk by HIV-U. Infants at six weeks of age on average are expected to consume ≥700g/day of breast milk per day (de Costa *et al.*, 2010). Slightly more than half of each of the groups of infants did consume above this amount. As shown in Table 5.5, there were no odds for the difference in the two groups in proportion of those who consumed ≥700g/day (aOR: 0.772, 95% CI: 0.287-2.071, p=0.60). About 90% of each of the group of infants consumed amount expected to be consumed by a 6 months old infant (800g/day) and there were no odds for the difference among the two groups (OR: 0.136 95% CI: 0.15-1.021).

Between 6 weeks and 6 months of age, the breast milk intake of both groups increased by 244g/day for HIV-EU and 251g/day for HIV-U. The increases were comparable (p=0.96) (not shown in the tables).

In each of the groups, no sex differences in the amount of breast milk consumed was detected. Overall, the males consumed 734.1g/day compared to the females who consumed 705.4g/day (p=0.175) at 6 weeks postpartum. At 6 months of age, males and female consumed 979.4 and 944.7g/day respectively (p=0.094).

Table 5.4: Breast milk intake of infants at 6 weeks postpartum and 6 months of age

Variable	Value or proportion		
	HIV-EU (n=60)	HIV-U (n=62)	P-value*
6 weeks postpartum			
Breast milk intake (gd^{-1} ±SD)	717.0 ±113.0	712.6 ±121.0	0.86
Breast milk intake ($gkg^{-1}d^{-1}$ ±SD)	154.8 ±29.3	152.5 ±22.6	0.63
Non-human milk water intake (gd^{-1} ±SD)	78.1 ±80.5	131.4 ±162.7	0.024
% consuming ≥700 gd^{-1}	55.0	55.6	0.87
6 months after birth			
Breast milk intake (gd^{-1} ±SD)	960.8 ±121.1	963.2±107.9	0.91
Breast milk intake ($gkg^{-1}d^{-1}$ ±SD)	136.7±17.0	136.0±15.0	0.80
Non-human milk water intake (gd^{-1} ±SD)	75.4 ±117.7	109.6 ±127.5	0.13
% consuming ≥800 gd^{-1}	90.0	90.3	0.95

*For continuous variables, students t-test for independent samples at α= 0.05, for categorical variables, Phi and Cramer's V statistics at α= 0.05

Table 5.5. The odds of consuming below the expected amounts of breast milk and <25g/day of non-human breast milk water

Variables	Adjusted Odds Ratio-aOR (lower limit-upper limit)$^{\infty}$	P-value
6 weeks		
Breast milk intake ≥700g/day	0.772 (0.287-2.071)	0.60
Non-human milk water intake below 25g	0.643 (0.198-2.086)	0.46
6 months		
Breast milk intake ≥ 800g/day	0.136 (0.15-1.021)	0.075
Non-human milk water intake below 25g	0.786 (0.275-2.252)	0.66

$^{\infty}$Adjusted for maternal age, maternal education, psychosocial status, household food security, season, infant sex and infant birth weight.

Among the HIV-positive mothers, those on ART and those not on ART had comparable breast milk output at 6 weeks (734.9g/day for ART and 701.3 g/day for non-ART; p=0.28) and at 6 months (961.1g/day for ART and 964.7; p=0.91) (Table 5.6). The two groups differed with ~34g/day at 6 weeks and ~4g/day at 6

months of age of infants. The difference between mothers with CD4≤350 cellls/mm^3 and CD4>350 cellls/mm^3 at 6 weeks postpartum were ~39g/day and zero (0.1) at 6 months, with the two groups being comparable (p=0.32 at 6 weeks and 0.99 at 6 months).

Table 5.6: Breast milk intake among HIV-positive mothers by ART and CD4 count

Maternal status$^{\infty}$	**6 weeks**	**6 months**
On ART	734.9 ±121.6	961.1 ±117.6
Not on ART	701.3 ±98.9	964.7 ±16.2
p-value	**0.28**	**0.91**
CD4≤350 cells/mm^3	695.2 ±130.3	965.3 ±80.9
CD4>350 cells/mm^3	734.3 ±120.4	965.4 ±133.1
p-value	**0.32**	**0.99**

$^{\infty}$n for on ART =35, non-ART=22. CD4<350, n=15 and for CD4>350= 32

5.4.3 Determinants of breast milk intake of HIV-uninfected infants

Multivariate analysis (multiple regressions) was conducted for between the amounts of breast milk consumed and the various maternal and infant factors as shown in Table 5.7. All the study infants irrespective of the study group were included in the analysis (n=121). In the regression model, maternal HIV status, maternal education and maternal age were controlled for (considered as covariates). Maternal Body Mass Index (r=0.247 for breast milk intake at 6 weeks) and fat free mass (r=0.270 and 0.365 at 6 weeks and 6 months respectively) were positively associated with breast milk intake. At 6 weeks postpartum, there was more likelihood for those not experiencing infections two weeks preceding the day of baseline saliva collection (for breast milk intake measurements) to have more breast milk output. The infection-breast milk output association was not significant at 6months after birth (p>0.05).

Infant birth weight was significantly associated with breast milk intake at 6 weeks (r=0.345) but not at 6 months (r=0.344) of the age of infant. Infant weight was associated with the intake at both points in time (r=0.486 and 0.557 at 6 weeks

and 6 months respectively). Infant sex and illnesses were not associated with breast milk intake.

Table 5.7: Multiple regressions between breasts milk intake of HIV-uninfected infants and maternal and infant factors

	Coefficient of correlations r		
	6 weeks	**6 months**	**Change between 6month and 6 weeks**
Maternal factors			
Primigravidae	0.181	0.132	0.184
Marital status	0.169	0.132	0.166
Body Mass Index	0.247*	0.200	0.166
Fat free mass	0.270*	0.365*	0.208
Household food security	0.188	0.132	0.168
Season	0.165	0.207	0.229
Negative psychosocial condition[∞¥]	0.174	0.183	0.222
Employment status	0.172	0.215	0.234
Infant factors			
Sex	0.207	0.189	0.164
Birth weight	0.345*	0.344	0.196
Birth order	0.165	0.134	0.171
Weight	0.486*	0.557*	0.176
Illnesses[∞]	0.174	0.149	0.166

*The relationship is significant ($p<0.05$)
[∞]Reported two weeks preceding the day 0 of measurements of breast milk intake
[¥]Any form of psychosocial status reported

5.5 Discussion

Maternal HIV status, HIV staging and treatment had no effect on breast milk intake. However, maternal HIV status influenced consumption of non-breast milk fluids. Maternal nutritional status (BMI and fat free mass) and infant size strongly influenced breast milk intake.

The amount of breast milk consumed by infants within the exclusive breastfeeding age bracket is a direct depiction of immunological benefits obtained from breast milk. The data generated in the present study thus indicate that infants maternally exposed to HIV and the unexposed receive same levels of these benefits. The amounts consumed by HIV-EU are comparable to that of HIV-U and are approximately the amounts expected to be consumed by infants at their

respective ages as reported by da Costa *et al.,* (2010). It occurs that the fear for transmission of the virus does not deter HIV-positive mothers to breastfeeding - and this may be attributed to the counselling on the reduced risk of transmission of virus to the infant and the benefits of breastfeeding. Most mothers (~60%) were also on combination ART (Tenofovir- Effavirenz combination or Tenofovir-Effavirenz- nevirapine combination) and majority (~77%) had CD4 cell counts of >350. ART ensures that these HIV-positive mothers are less affected by opportunistic illnesses and thus able to effectively care for and breastfeed their infants. Infections (such as subclinical mastitis) have been shown to affect breast milk output. High levels of breast milk RNA viral load are associated with sub-clinical mastitis (Wilumsen *et al.,* 2003) and ART started during pregnancy or postpartum suppresses breast milk HIV-1 RNA (Shapiro *et al.,* 2005; Chung *et al.,* 2008). This may explain why the observation of reduced breast milk with the occurrence of sub-clinical mastitis in dairy cows (Shuster *et al.,* 1995) is not consistent with the findings of this study.

It is known that the factors that determine (drive) breast milk intake of infants could be maternal or infant related. These factors have been determined earlier when the use of test-weight was the common method that was the used in measuring breast milk (Dewey *et al.,* 1999; Brown *et al.* 1986; Stuff *et al.* 1986). In the advent of use of isotopic technique, test-weighing technique has been unpopular. The present study used isotopic techniques and some breast milk intake determinants are reported. Maternal BMI which is an index of weight and height is positively associated with breast milk intake. This is consistent with the results of Brown *et al.* (1986) but not of Dewey *et al.* (1999) and Stuff *et al.* (1986). Maternal lean mass (as estimated by deuterium oxide technique) was associated with breast milk output. Previous associations used maternal body composition using the skin fold thickness as proxy for body composition. Dewey *et al*. (1999) for instance did not find any association between maternal triceps skinfold and breast milk output. Birth weight has been long been associated with breast milk intake (Butte *et al.,* 1984; Dewey *et. al.,* 1999) and this present study

confirms this. The association of weight is also know through the pathway of increasing in infant 'suckling power', but this may also be reverse and/or cyclic association – where the high breast intake lead to greater increase in weight and increased weight lead to higher intakes of breast milk.

5.6 Conclusions and Recommendations

Exposure to HIV does not affect the amount of breast milk a Kenyan HIV-uninfected infant 6 months old and below consumes. Interventions that ensure normal maternal body mass index, maternal lean mass, infant size (birth and current weight) are important in increasing breast milk intake of HIV-uninfected infants.

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CHAPTER SIX

6 COMPARISON OF LINEAR GROWTH OF KENYAN HIV-NEGATIVE INFANTS BORN TO HIV-INFECTED MOTHERS IN RESOURCE POOR SETTING TO THAT OF HIV-UNEXPOSED INFANTS BY 6 MONTHS OF AGE

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6.1 Abstract

Objectives: To compare the growth of HIV-uninfected infants born of HIV-positive mothers and those born of HIV-negative mothers.

Methods: This was part of a study to compare breast milk intake of HIV-exposed, uninfected and HIV-unexposed using the deuterium oxide dose-to-mother technique. Seventy-two (72) HIV-1 positive and 66 negative mothers attending Maternal and Child Health Clinic, with their HIV-uninfected infants were recruited at 6 weeks and followed-up to 6 months of age. Maternal and infant anthropometries (height/length, weight and MUAC) were measured at recruitment and follow-up.

Results: There were significant difference between HIV-exposed uninfected and HIV-unexposed infants in length-for-age Z scores (LAZ) and weight-for-length Z scores (WAZ) at 6 weeks postpartum ($p=0.011$ and 0.028 respectively). At 6 months of age, there were no significant differences in mean LAZ, weight-for-age Z scores and WLZ ($p=0.154$, 0.532 and 0.631, respectively) between the two groups. There were no odds for the proportion of those with <-2 LAZ, WAZ and WLZ being greater in any of the two groups at both 6 weeks and 6 months (aOR<1 and/or $p>0.05$). Among the infants born to HIV-positive mothers, growth in length and weight of those whose mothers were on ART did not differ from non-ART at 6 weeks postpartum and 6 months of age. The maternal CD4 cell

count did not also affect linear growth. Between week 6 and month 6, change in HIV-uninfected infants linear growth was positively correlated with breast milk intake at 6 months (r=0.371) and the change in milk intake between week 6 and month 6 (r=0.328).

Conclusions: Poorer growth earlier in the life of HIV-EU compared to HIV-U is corrected for by 6 months after birth. Exclusive breastfeeding and on demand (to achieve higher breast milk intakes) is important for better linear growth for HIV-uninfected infants.

Key words: Growth, body composition, HIV-exposed uninfected infants, HIV-unexposed infants

6.2 Introduction

With the scaling up of Prevention of Mother to Child Transmission, majority in sub-Sahara Africa, about 95% of the infants born of HIV-positive mother are therefore themselves HIV-free at birth (WHO, 2012). The effect of maternal HIV exposure on the well-being of infants has lately been an important public health concern. This is due to the rise in their numbers and their heightened vulnerability to negative health and nutrition outcomes. It is now known that infant freedom from HIV does not necessarily guarantee sound growth of the infants (Sugandhi *et al.*, 2013; Filteau, 1999). Infant exposure to maternal HIV may influence linear growth of their infants. For instance, HIV-positive mothers were more likely to experience sub-clinical mastitis (Kasonka *et al.*, 2006) and this clinically undetected mastitis was associated with the infants growth in length (Kasonka *et al.*, 2006; Gomo *et al.*, 2003). Part of altered linear growth among the HIV-exposed uninfected infants may be due to modified infant feeding practices for infants born of HIV-positive mothers (Filteau, 1999). But current recommendations for all infants (irrespective of the infant or maternal HIV status) are to be exclusively breastfed for the first 6 months of life (WHO, 2010). Nevertheless, since the advent of this WHO 2010 recommendation, there is paucity of data on how well the HIV-uninfected infants born of HIV-positive mothers grow in comparison to those born of HIV-negative mothers. This study

thus investigated the effects of HIV exposure on growth of HIV-uninfected Kenyan infants.

6.3 Methods

6.3.1 Study design and setting

This study was part of a research designed to measure the breast milk output among the HIV-1 negative and positive mothers with HIV-1 negative infants using stable isotope technique (deuterium oxide dose-to-mother technique). Infants' anthropometric measurements were taken at recruitment (6 weeks post-partum) and at 6 months of age of infants. The study was set at the Maternal and Child Health (MCH) Clinic of Siaya County Referral Hospital in Western Kenya. Mother-infant dyads were recruited into the study as they attended post-natal care between February 2014 and September 2014. Siaya County is in the Lake Region of Kenya where HIV rates are highest. Siaya County inhabited by predominantly rural population with a HIV prevalence of 15.9% among adult men and 23.3% among adult women. These rates are way above the national average of 5.6% and 7.6% among adult men and women respectively (NAAC, 2015).

6.3.2 Sample size determination

The sample size was computed based on the objective of comparing breast milk intake of HIV-uninfected infant born of HIV-1 positive and negative mothers, as explained by Kirkwood and Sterne (2003) as explained in section 3.2 of this manuscript.

6.3.3 Sampling

It was estimated that on a busy day of the week, about 20 mother-infant dyads were expected at the post-natal clinic. The target was to recruit half of post-natal attendance each day. Alternate mothers were thus considered for the study. The first mother to line up for the antenatal care was consented and second was not considered unless the preceding mother declined to participate in the study. Seventy-two (72) HIV-1 positive and 66 negative mothers were recruited into the study at 6 weeks post-partum. Retesting for HIV status was repeated at 6 months where the 66 HIV-1 positive and 63 negative mothers were successfully followed up. The rest were lost to follow-up (moving away from the study area).

6.3.4 Exclusion criteria

The following mothers-infant dyads were excluded from the study: those with infants having <2500g birth weight, with preterm infants, those not able to breastfeed and mothers or infants showing signs of chronic illnesses. To minimise loss to follow-up, those who intended to move far away from their then current locales 6 months from the time of recruitment were also excluded.

6.3.5 Maternal and infant HIV testing

Maternal HIV status was done using the antibody tests and infants were tested with HIV-1 DNA polymerase chain reaction (PCR) as explained in section 3.5.

6.3.6 Maternal and infant anthropometry

Anthropometric measurements were taken using the Standard Operation Procedures described in the Annex 3.1.

Height/length: Mother height and infant length was determined using the Infant/Child/Adult ShorrBoard (79" x 1/8" / 200 x 0.1 cm) measured to the nearest 0.1 cm.

Weight: Maternal weight was determined by using Camry weighing scale (Model EB 9318), and recorded to the nearest 0.1 kg, while the nude weight of infants was measured using the Salter Electronic Baby (maximum 25kg).

MUAC: ShorrTape (65 cm x 0.1 cm) and Shorr Child MUAC Tape (26 cm x 0.1 cm) were used for determining the maternal and infant MUAC respectively.

6.3.7 Other data collected

Maternal socio-demographic data (at week 6) were collected using a structured questionnaire. Gestational age of the infants, date of birth and birth weight were obtained from the mother and child clinic cards, and confirmed by mother recall. The study clinic based the determination of the gestational age on mother's last reported menstrual period. Maternal and infant illnesses were based on the observed sicknesses two weeks prior the interview. Breast milk intake and exclusive breastfeeding practice was determined as explained in section 4.3.4 and 4.3.5 in chapter 4.

6.3.8 Data cleaning and statistical analysis

Data was entered in EPI6 and cleaned in MS-Excel™ before being transferred to Statistical Package for Social Scientists (SPSS) version 20 for analysis. Standard normal deviates (Z scores) were computed using the ENA for Smart 2011 using WHO Child Growth Standards (WHO Multicenter Growth Reference Study Group 2006). The WHO Global Database on Child Growth and Malnutrition uses a Z score cut-off point of <-2 SD to classify low weight-for-age (WAZ), low length-for-age (LAZ) and low weight-for-height (WHZ) as moderate and severe undernutrition, and <-3 SD to define severe undernutrition. Differences in Z scores for LAZ, WAZ and WAZ between two groups were compared using the students t-tests at $\alpha = 0.05$ and p-values <0.05 reflected statistically significant difference. A linear regression between the growth and infant and maternal variables (shown in Figure 2.1) was run to identify potential confounders for which adjustment was necessary. Variables which yielded $p<0.1$ were controlled for in analyses. Some variables identified in literature were also included. For the linear regression analysis, all infants were grouped together as HIV-uninfected infants to identify some correlates of growth. Those with only one point measurement were excluded. One hundred and twenty three (126) cases were considered for this analysis that is those who were measured both at weeks 6 and 6 months of age points. For analysis involving breast milk intake, likewise only infants whose measurements were done both at week 6 and month 6 were considered (n=121). The change in Z scores between 6 weeks and month 6 of the infant life were correlated with maternal and infant variables. Statistical significances were tested at both 0.01 and 0.05 levels.

The odds ratios for proportion of infants being <-2 Z scores among the two groups were adjusted for maternal height (due to the reported link- Cooper *et al.*, 2001; Varela-Silva *et.al.,* 2009). Infant sex was also adjusted for due to sex variations in growth as has been reported (Baig-Ansari *et al*., 2006; Wamani *et al.,* 2007). Maternal % fat mass, infant birth weight and breast milk intake were also adjusted due to the positive correlation with infant LAZ ($p<0.1$) at

recruitment. Maternal age and education were also adjusted for due to the difference between the HIV-positive and negative mother in these variables.

6.3.9 Ethic considerations

Ethical approval was secured from Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UON/ERC). Only the mothers who consented were recruited and followed-up.

6.4 Results

6.4.1 Maternal and Infant Characteristics at recruitment (6 weeks postpartum)

As depicted in the Table 6.1, the recruited HIV-1 positive mothers were younger than their HIV-negative counterparts (p=0.002). Mean maternal height, Body Mass Index (BMI) and MUAC were comparable at recruitment and follow-up. Majority of the mothers were within the normal ranges BMI and MUAC for nourished mothers.

Table 6.1: Maternal and child general characteristics at 6 weeks postpartum

Maternal characteristics	**HIV-1 positive (n=72)**	**HIV-1 negative (n=66)**	**p-value**$^{\infty}$
Mean age (years ±SD)	28.8 ±6.0	25.4 ±6.0	0.002
Height (m ±SD)	160.4 ±5.9	160.6 ±6.0	0.82
BMI (kgm^{-2} ±SD)	22.7 ±3.5	22.7 ±3.3	0.91
% BMI <18.5cm	2.8	7.6	0.21
MUAC (cm ±SD)	26.7 ±3.3	26.9 ±3.2	0.72
% MUAC <21cm	1.4	1.5	0.95
CD4 cell count (cells/mm^3)	492.7 ±297.7	-	-
%CD4 cell count <350cells/mm^3)	26.1	-	-
Proportion of ART	59.1%		
Infant characteristics	**HIV-EU (n=72)**	**HIV-U (n=66)**	**p-value**∞
Sex (% female)	61.3	45.6	0.059
Mean gestational age at birth (months ±SD)	8.7 ±1.0	8.7 ±0.5	0.72
Birth order ±SD	3.4 ±1.9	2.9 ±1.8	0.14
Mean age (months ±SD)	1.5 ±0.1	1.4 ±0.3	0.087
% with gastrointestinal infections[¥]	55.6	66.7	0.18
% with respiratory infections[¥]	33.3	36.4	0.71
% with both gastrointestinal and respiratory infections[¥]	23.6	28.8	0.49

$^{\infty}$For continuous variables, p values based on students t-test for independent samples at α= 0.05.
For categorical variables, Phi and Cramer's V statistics used at α= 0.05 used.
[¥] Maternal recall of illnesses experienced 2 weeks preceding the interview

About 26.1% of HIV-positive mothers had CD4 cell count of <350cells/mm^3 and ~two-thirds of them were on Antiretroviral (ART) drugs. HIV-EU and HIV-U

infants were comparable in sex composition, gestational age (none were pre-terms), parity, age at recruitment, and in morbidity pattern preceding recruitment.

6.4.2 Growth of infants at 6 weeks postpartum and 6 months of age

Table 6.2 compares the mean LAZ, WAZ and WHZ of HIV-EU and HIV-U infants at 6 weeks postpartum and when they were 6 months old. At recruitment and follow-up, none of the two groups had mean LAZ, WAZ and WLZ of < -2. At 6 weeks postpartum, HIV-U had greater LAZ and WAZ (p=0.011 and 0.028 respectively) compared to HIV-EU. The two groups of infants had comparable LAZ, WAZ and WLZ at 6 months of age. The difference in LAZ between the two groups was 0.5 at 6 weeks postpartum and 0.3 at 6 months of age – depicting reduction in differences over time. For WAZ, the difference increased from 0.30 at week 6 to 0.9 at month 6. For WAZ, the difference declined from 1.7 to 0.9 between week 6 and month 6.

Table 6.2: Infant growth at 6 weeks post-partum and 6 months of age

Z scores	Groups	Mean Z scores (±SD)	
		6 weeks	**6 months**
LAZ	HIV-EU	-1.1 ±1.0	-1.2 ±1.3
	HIV-U	-0.6 ±1.3	-0.9 ±1.4
	P-value	**0.011**	**0.15**
WAZ	HIV-EU	0.3 ±1.0	-0.5 ±1.3
	HIV-U	-0.05 ±1.1	0.4 ±1.3
	P-value	**0.19**	**0.53**
WLZ	HIV-EU	1.1 ±1.1	0.5 ±1.3
	HIV-U	0.6 ±1.3	0.4 ±1.4
	P-value	**0.028**	**0.63**

P-values were based on students t-test for independent samples at $\alpha = 0.05$. At 6 weeks postpartum, n for HIV-EU =72 and HIV-U =66. At 6 months of age, n=63for both HIV-EU=63 and for HIV-U.

Table 6.3 depicts the proportion of infants with <-2 Z scores (LAZ, WAZ, WLZ). The Odds Ratios (OR) were adjusted for maternal height, maternal % fat mass, maternal education, maternal age, infant sex, infant birth weight and breast milk intake. Majority of the study infants were within the normal (>-2 Z scores). There were no odds for proportion of infants with <-2 to be higher in any of the groups (p>0.05). The prevalence of stunting for both groups at 6 weeks and 6 months

were higher than the national average of 10% (GOK, 2014). This was the same case for the underweight of which the national average stands at 3.7%. The national wasting average also stands at 3.7%.

Table 6.3: Prevalence of infants with Z score <2

	Prevalence (%)		**aOR$^{\infty}$**	**P-value**
6 weeks	**HIV-EU (n=72)**	**HIV-U (n=66)**		
Prevalence of LAZ <-2 (stunting)	16.7	13.6	0.530 (0.151-1.89)	0.32
Prevalence of WAZ<-2 (underweight)	5.6	6.1	2.615 (0.057-119.7)	0.62
Prevalence of WLZ <-2 (wasting)	0.0	3.1	$2.5*10^{6}$(0-00)	1.00
6 months	**(n=63)**	**(n=63)**		
Prevalence of LAZ <-2 (stunting)	18.6	17.9	1.434 (0.365-5.641)	0.60
Prevalence of WAZ<-2 (underweight)	10.2	10.4	0.728 (0.102-5.180)	0.75
Prevalence of WLZ <-2 (wasting)	3.4	7.5	0.378 (0.019-7.407)	0.52

$^{\infty}$Adjusted Odds Ratio – adjusted for maternal height, maternal % fat mass, maternal education, maternal age, infant sex, infant birth weight and breast milk intake

Among the infants born of HIV-1 positive mothers, no differences were observed between infants born of mothers on ART and those not on ART ($p>0.05$) (Table 5.4). No differences were also observed between infants with mothers of CD4 cell count <350 and those with >350cells/mm^3 ($p>0.05$) (Table 6.4)

Table 6.4: Growth of infants among HIV-1 exposed infants by maternal ART and CD4 cell count

Maternal status	6 weeks Z scores (±SD)			6 months Z scores (±SD)		
	LAZ	WAZ	WLZ	LAZ	WAZ	WLZ
On ART (n=41)	-1.1±1.0	-0.4±0.8	1.0±1.0	-1.1±1.5	-0.5±1.4	0.4±1.4
Not on ART (n=28)	-0.9±1.1	-0.06±1.1	1.2±1.2	-1.2±1.2	-0.5±1.2	0.5±1.3
p-value$^{\infty}$	**0.42**	**0.16**	**0.44**	**0.95**	**0.86**	**0.86**
CD4≤350 cells/m^{-3} (n=17)	-1.1±.94	-.4±1.1	0.9±1.1	-1.6±1.7	-0.6±1.3	0.8±1.4
CD4>350 cells/m^{-3} (n=36)	-1.0±1.1	-0.2±1.0	1.0±1.2	-0.8±1.1	-0.3±1.3	0.4±1.4
p-value$^{\infty}$	**0.72**	**0.62**	**0.77**	**0.70**	**0.45**	**0.36**

P-values based on students t-test for independent samples at $\alpha = 0.05$.

6.4.3 Correlates of growth at 6 weeks and 6 months of age among HIV-uninfected infants

Table 6.5 shows the correlates of change in Z scores at 6 weeks and 6 months. Change in LAZ was positively (and significantly) correlated with breast milk intake at 6 months and change in breast milk intake. It was surprising that LAZ significant negative correlation with birth weight. The positive correlations between change WAZ and maternal % fat mass, infant birth weight, Z score at 6 weeks, breast milk intake at 6 months and change in breast milk intake were significant. The correlations between change WLZ and maternal height (negative correlation), Z score at 6 weeks (positive), breast milk intake at 6 months (positive) and change in breast milk intake (positive) were significant.

Table 6.5: Correlates of change in Z scores among the HIV-uninfected infants (using Pearson correlation)

Variables	Coefficient of correlations (r)		
	LAZ	**WAZ**	**WLZ**
Maternal variables			
HIV status	0.038	0.07	-0.155
Age	0.097	-0.016	0.0576
Body Mass Index	0.071	0.282	0.201*
Fat free mass	0.167	0.104	-0.031
% fat Mass	0.080	0.317**	0.151
Height	0.057	0.065	-0.186*
ART status∞	-0.009	0.023	0.023
CD4 cell count$^{\infty}$	0.258	0.110	0.131
Infant variables			
Sex	0.086	0.107	0.040
Birth weight	-0.240*	0.465**	-0.006
Birth order	0.050	0.08	-0.056
Respective Z score at 6 weeks	-0.48**	0.596**	0.383**
Breast milk intake at 6wk	-0.107	-0.078	0.033
Breast milk intake at 6mo	0.371**	0.603**	0.410**
Change in breast milk intake ¥	0.328**	0.467**	0.259**
Change in Non-breast milk water intake¥	-0.085	-0.053	0.035
EBF at 6wk$^{\Omega}$	0.061	0.154	0.138
EBF at 6mo¥	0.001	0.116	0.164

∞Only for HIV-1 positive mothers where n=59
¥As determined by deuterium oxide dose-to-mother technique. Change is between 6 weeks and 6 months values $^{\Omega}$Infants who consumed $\geq$25g of non-human milk water were considered to be exclusively breastfed.
*Pearsons correlation is significant at the 0.05 level ($p<0.05$)
**Pearsons correlation is significant at the 0.01 level ($p<0.01$)

In order to test the influence of HIV on the changes in growth due to variations in breast milk intake, 2 models were considered (Table 6.6). In a multivariate analysis, multiple linear regressions between change in Z scores and change in breast milk intake at week 6 and at month 6 were adjusted for maternal age, education level, psychosocial status, food security, season, infant sex and birth weight (model 1) and in the second model, all in the model 1 with an addition of maternal HIV status were adjusted for. Only in the univariate was the change in

WAZ was positively associated with change in breast milk intake (r=0.240). There was no observed changes in coefficient of correlation r between model 1 and model 2 indicating that HIV was not a significant factor in the association between the change in Z score and change in breast milk intake.

Table 6.6: Regression of change in the Z scores and change in breast milk intake among HIV-uninfected infants

	Coefficient of correlation- r		
Indicator	**Unadjusted**	**Model 1**	**Model 2**
LAZ	0.179	0.274	0.274
WAZ	0.240*	0.265	0.266
WLZ	0.042	0.275	0.290

*p-value<0.05
Model 1: adjusted for maternal age, education level, psychosocial status, food security, season, infant sex and birth weight
Model 2: Adjusted for all indicators in model 1 plus maternal HIV status

6.5 Discussion

Compared to the HIV –unexposed, HIV -uninfected infants exposed to the HIV had lower (and significantly different) linear growth at 6 weeks post-partum, but the difference was not significant at 6 months after birth. Maternal intake of ART did not affect the growth infants so was the stage of HIV infection as depicted by the CD4 cell count. Increases of breast milk intakes between 6 weeks and 6 months after birth is positively correlated with change in Z scores (LAZ, WAZ and WLZ). Maternal HIV status is not an important factor in the association between growth and breast milk intake.

A review of studies on maternal HIV and pregnant outcomes revealed that the maternal HIV infection has negative effects on the pregnancy outcomes including intrauterine growth retardation (which leads to low birth weight) and preterm births (Brocklehurst *et.al*., 1998) which affect early growth of the infants. The present study was designed to eliminated the influence of these factors by compared the growth of HIV-EU and HIV-U of infants who were to term, with birth weight $\geq$2,500g and with no chronic illnesses. The incidences of low birth weight or preterm births have declined during an era of increased maternal antiretroviral therapies (Schulte *et al*., 2007). By extrapolation, it may be argued

that in early life the growth of HIV-EU and HIV-U should be similar. This was not the case for the Kenyan infants in the study at 6 weeks post-partum where HIV-EU had lower linear growth compared to HIV-U at 6 weeks. This is consistent with the finding among 3 months old infants from Democratic Republic of Congo (Barley *et al*., 1999) and among 6 weeks old Zambian infants (Masaka *et al*., 2007). As the infants reach 6 months of age, the present study indicates that differences in LAZ between the two groups diminish. It is apparent from this study that infants maternal exposed to HIV-1 status are catching up with the unexposed ones by 6 months of age of infants. This may not be the actual point of catch up since this present study did only measure growth at two points. Ramokolo *et al*. (2014) observe the similarity in growth between the two groups at 3 weeks and this continued to 25-36 weeks. There seems to be intrinsic factors that limit early growth among the HIV-EU, and these factors become inconsequential as the infant grows older. This phenomenon is positive and favourable for the HIV-EU.

Amount of breast milk taken can explain the similar linear growth at 6 months. Deven *et al*. (2010) and Mwiru *et al*., (2011) have indicated that optimal early feeding practices ameliorate the effect of being born to an HIV-infected mother. In low resource setting, the effects of sound breastfeeding practice (feeding exclusively and on demand) leads to increased breast milk taken by the infant. In this present study, amount of breast milk was positively correlated with infant growth at 6 months of age. From the same study groups, the amount of milk taken by HIV-EU and HIV-U were not different at both week 6 and month 6 (Chapter 4).

The study subjects were drawn from the hospital set up where PMTCT services are offered and HIV-positive mothers are constantly counselled, offered treatment, followed up and supported to breastfeed. There is a possibility that this also partly contributed to the similarity in growth of the two study groups. In a non-resource poor population (where HIV-positive mothers receive the needed services) the uninfected children have been reported to have normal growth,

which is unaffected by exposure to maternal HIV infection (Newell *et al*., 2003). Majority (~60%) of the HIV-positive mothers are also on ART and almost all were in one or the other support group. Tenofovir- Effavirenz combination or Tenofovir- Effavirenz- nevirapine combination) and antibiotics (septrin) were provided to HIV-positive mothers. The provision of ARVs assures low maternal morbidity and reduced infant exposure to infections and this may partly explain why by 6 months, the growth of HIV-EU is not different from that of HIV-U. However, no differences were found in growth among infants whose HIV-positive mothers are on ART and as compared to non-ART. This is consistent with the findings of Culnane *et al*.,(1999) that there were no adverse effects in length and weight in HIV-uninfected children exposed to ART.

6.6 Conclusions and Recommendations

Poorer growth earlier in the life of HIV-EU compared to HIV-U is corrected for by 6 months after birth. Exclusive breastfeeding and on demand (to achieve higher breast milk intakes) is important for better linear growth for HIV-uninfected infants.

6.7 References

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CHAPTER SEVEN

7 BODY COMPOSITION OF KENYAN HIV-EXPOSED UNINFECTED INFANTS AT 6 MONTHS OF AGE USING DEUTERIUM DILLUTION METHOD AND VALIDATION OF SOME INFANT BODY COMPOSITION PREDICTION EQUATIONS

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7.1 Abstract

Background: The body composition in early life has significant health effects in the future life health outcome including obesity, hypertension, type 2 diabetes, cardiovascular diseases and stroke.

Objective: To compare body composition of HIV-exposed, uninfected infants with that of HIV-unexposed infants at 6 months of age.

Methods: Body composition for 6 months old 32 HIV-exposed, uninfected and 32 HIV –unexposed infants was measured using deuterium –dose-to the infant technique. Pre-dose saliva sample was collected from the infant after which a deuterium oxide dose (0.5g/kg/body weight) was administered and post-dose saliva samples collected at 3 and 4 hours post-dosing. Total Body Water (TBW) from the FTIR measurement was converted to lean mass using standard equations and assumptions. The Bland and AItman pair-wise comparison method was used to evaluate the agreement of % fat mass (as determined by deuterium dilution method) and prediction equations using infant skin fold thicknesses, Mid-Upper Arm Circumference and infant age.

Results: HIV-exposed, uninfected and the HIV-unexposed had comparable fat free mass (5.7kg for HIV-EU and 5.9 kg for HIV-U, p=0.10), fat mass (1.6kg for HIV-EU and 1.6kg HIV-U, p=1.0), % fat mass (22.3% for HIV-EU and 21.3% for HIV-U, p=0.34), fat free mass index (14.7 kgm^{-2} for HIV-EU and 14.5 kgm^{-2} for

HIV-U, p=0.73) and fat mass index (4.3 kgm^{-2} for HIV-EU and 4.0 kgm^{-2} for HIV-U, p=0.35). Among infants born of HIV-positive mothers, those whose mothers were on ART had lower free fat mass (5.4kg verses 6.0kg, p=0.018), conversely higher % fat mass (24.0% versus 19.3% for non-ART, p=0.004) and lower free fat mass index (14.2kg verses 16.0kg for non-ART, p=0.042). Infants of mothers with CD4 cell <350 cells/mm^3 had comparable lean and fat mass with those born of mothers with CD4 cells $\geq$350 cells/mm^3. Amount of breast milk consumed by HIV-uninfected infants was significantly and positively correlated with infant lean mass (r=0.227 at 6 weeks, r=0.645 at 6 months and r=0.265 for the change in breast milk intake between 6 weeks and 6 months). Equations for predicting the body mass index either over or under-estimated the % fat as determined by the deuterium dilution method. The Bland and AItman pair-wise comparison method depict biases between the averages and differences between the % fat mass a predicted by skinfold thickness and as determined by deuterium dilution.

Conclusions: Although in overall infants of HIV positive mothers have similar lean mass to that of HIV-negative mother by 6 months after birth, ART passed from the mother to infant through breast milk may promotes infant adiposity. No equation reliably predicts the deuterium dilution determined body composition for Kenyan infants, necessitating the formulation of Kenya-specific body composition prediction equations.

Key words: Body composition, HIV-exposed uninfected, HIV –unexposed, % fat mass predictor equations

7.2 Background

Potential factors and pathways that may influence growth of HIV-exposed, uninfected (HIV-EU) infants have been reported (Sugandhi *et al*., 2013; Filteau, 1999). There is however paucity of data on the effect of HIV exposure on the infant body composition (the proportion of water, fat, bone and muscles). Among other negative health outcomes later in life, obesity, hypertension, type 2 diabetes,

cardiovascular diseases and stroke are linked to body composition early in life (Wells *et al*., 2007). Additionally, with the advent of standard treatment for HIV (WHO, 2010), all Kenyan mothers are required to be on combination antiretroviral (ART) as soon as they are tested positive. There is however paucity of data on the potential effects of the maternal ART and the stage of HIV progression on infant body composition. Due to the importance of the determination of body composition, different methods have been devised for the measurements including dual energy X-ray absorptiometry (DEXA), deuterium dilution methods of determining total body water, bioelectric impedance analysis and air displacement plethysmography. In sub-Sahara Africa, these methods are expensive and out of reach of many researchers and health clinics. This has given rise to the development of a number of prediction equations aiming at estimating % fat mass using simple anthropometric measurements. This present study analyzed the body composition of two groups of 6 month old HIV-uninfected infants (HIV-exposed and -unexposed) using deuterium dilution method. This also provided an opportunity for validating the available equations for predicting Kenyan infants body composition using skinfold thickness measurements.

7.3 Methods

7.3.1 Study design and setting

Body composition measurements using deuterium dilution method was determined cross-sectionally for six months old infants. The study was set at the Maternal and Child Health (MCH) Clinic of Siaya County Referral Hospital in Western Kenya. HIV-uninfected infants of 32 HIV-positive mothers and 32 HIV-negative mothers were systematically selected from those followed-up at 6 months after birth. Alternate mother-infant dyads returning for 6 month postpartum breast milk intake measurements were considered.

7.3.2 Exclusion criteria

The following mothers-infant dyads were excluded from the study: those with infants having <2500g birth weight, with preterm infants, those not able to

breastfeed and mothers or infants showing signs of chronic illnesses as determined by the research project clinician.

7.3.3 Maternal and infant HIV testing

Maternal HIV status was done using the antibody tests and infants were tested with HIV-1 DNA polymerase chain reaction (PCR) as explained in section 3.5, chapter 3.

7.3.4 Body composition measurements by deuterium oxide dilution method

To determine infants body composition, they were given a fixed standardized dose of deuterium labelled water which had been accurately weighed as explained by International Atomic Energy Agency (IAEA) in Vienna, Austria (IAEA, 2009). A pre-dose sample of 2ml of saliva was taken from the infant's mouth by using a passive cotton ball soaking collection method and marked as pre-dose (T0). Then 15 ml of deuterium oxide solution (2.5g deuterium and 12.5 ml of mineral water-equivalent to 0.5g/kg/body weight) was given to the infant orally via a syringe barrel taken. Post-dose saliva samples were taken at 2 hours and 3 hours. Infants were fasted for at least 30 minutes before the saliva samples were. All saliva samples were collected into a tightly capped cryogenic tube and kept in a freezer -20^{o}C awaiting transportation. Samples were then transferred in dry ice package to the Kenya Medical Research Institute (KEMRI) nutrition laboratories in Nairobi, Kenya. At this laboratory, enrichment of deuterium in saliva samples was determined using Fourier Transform Infrared (FTIR) spectrophotometer (Shimadzu, Vienna, Austria). Using the mean of deuterium enrichment based on the two post-dose samples, the dilution space and total body Water (TBW) were calculated as described by IAEA (IAEA, 2009). Fat free mass (lean mass) was calculated as: TBW/0.79 for both sexes. Fat Mass (FM) was computed by subtracting the FFM from the subject weight. Free Fat Mass Index (FFMI) and the Fat Mass Index (FMI) were computed by dividing individual infant's FFM and the FM by the squares of their respective height

7.3.5 Maternal and infant anthropometry

Maternal and infant height/length, weight and MUAC: These measurements were taken as explained in section 3.6.2 in chapter 3.

Biceps, triceps and subscapular and suprailliac: These skinfold-thickness measurements were done using Holtain skinfold calipers (Crymych, United Kingdom) to the nearest 0.1mm.

7.3.6 Prediction equations for estimating % fat mass

A list of 11 possible equations for predicting % fat mass were obtained from the available literature. Only three were screened to be suitable for predicting % fat mass for 6 months old infants. These were equations as developed by Bandana *et al*. (2010), Shailk and Dulip (2004) and Liu *et al*. (2010). As shown in Table 7.1, these equations were sex specific and used skin fold thicknesses, Mid-upper Arm circumferences and infant ages.

7.3.7 Statistical analysis

Data was entered in EPI6 and cleaned in MS-Excel^{TM} before being transferred to Statistical Package for Social Scientists (SPSS) version 20 for analysis. Standard normal deviates (Z scores) were computed using the ENA for Smart 2011 using WHO Child Growth Standards (WHO Multicenter Growth Reference Study Group 2006). Differences in Z scores for Length for Age (LAZ), Weight for Age (WAZ) and Weight for Length (WAZ) between two groups were compared using the students t-tests at $\alpha = 0.05$ and p-values <0.05 reflected statistical significance. An initial regression between infant lean mass and infant and maternal variables was run to identify potential confounders. Maternal % fat mass, infant birth weight and breast milk intake were also adjusted due to the positive correlation with lean mass ($p<0.1$). Some variables were also identified in literature as potential confounders and adjusted for. They were maternal height (Cooper *et al*., 2001; Varela-Silva et.al., 2009). Infant sex was also adjusted for due to potential sex variations (Baig-Ansari *et al*., 2006; Wamani et. al, 2007). Maternal age and education were also included as covariates due to the differences between the HIV-positive and negative mothers.

Table 7.1: Prediction equations for % fat mass using anthropometric measurements

Equation source	Prediction equation: % FM=	Group applied to	Method used
Males			
Bandana *et al*., 2010	-8.75 + 3.73×B + 2.57×S	6-24 months old	DD
Shailk and Dulip, 2004	5.304 + 0.269xT + 0.50xSS + 0.685xM - 0.063xA	Pre-school children	BI
Liu *et al*., 2010	1.21 x T+SS-0.008 x $(T+SS)^2$-1.7	Infants 8 weeks old	ADP
Female			
Bandana et.al, 2010	-69.26 + 5.76×B - 0.33×T^2 + 5.40×M + 0.01×A2	6-24 months old	DD
Shailk and Dulip, 2004	7.017 – 0.053xT + 0.201xSS + 0.765xM + 0.052xA	Pre-school children	BI
Liu *et al*., 2010	1.33 x T+SS-0.013 x $(T+SS)^2$-2.5	Infants 8 weeks old	ADP

S=Supraliac skinfold in mm, B=Bicept skinfold in mm, T=Tricept skinfold in mm,
SS=Subscapular skinfold in mm, A= Age in months and M=MUAC in cm
DD=Deuterium Dillution, BI= Bio-electric impendance, ADP= air displacement plethysmography

7.3.8 Ethics

Ethical approval was secured from Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UON/ERC). Only the mothers who consented were considered for the study.

7.4 Results

7.4.1 General characteristics

Body composition was assessed for infants who were term, had birth weights >2500g and did not show clinical signs of severe illnesses. Additionally Table 7.2 shows that the two groups of infants assessed had significant different birth orders (HIV-EU having higher birth order). The HIV-U infants had higher (and significantly different) lengths, length-for-age Z scores (LAZ), weight-for-age Z scores (WAZ) than the HIV-EU. The two groups had comparable skin fold thicknesses. Both the HIV-EU and HIV-U on aggregate were not moderately or severely malnourished (mean Z scores >-2). The two groups had comparable skin fold thicknesses ($p>0.05$) as shown in the Table 7.2

Table 7.2: Infant characteristics at 6 months of age

Characteristic	Proportion of value		P-value[∞]
	HIV-EU (n=32)	HIV-U (n=32)	
% female	56.3	59.4	0.80
Birth order	3.3 ±1.3	2.5 ±1.4	0.026
Birth weight in kg (±SD)	3.7 ±2.7	3.3 ±0.5	0.42
Length in cm (±SD)	61.9 ±3.01	63.7±3.1	0.021
Weight in kg (±SD)	6.8 ±0.9	7.3 ±0.9	0.045
LAZ (±SD)	-1.7 ±0.4	-0.8 ±1.4	0.010
WAZ (±SD)	-0.7 ±1.2	-0.06 ±1.0	0.016
WAZ (±SD)	0.7 ±1.1	0.7 ±1.0	0.90
Skin fold thicknesses in mm (±SD)			
Biceps	6.2 ±1.0	6.4 ±1.2	0.39
Triceps	8.1 ±1.2	8.6 ±1.6	0.16
Subscapular	7.7 ±1.3	8.3 ±1.7	0.18
Suprailliac	7.0 ±1.9	7.3 ±1.9	0.59

[∞]For continuous variables, p values based on students t-test for independent samples at α= 0.05. For categorical variables, Phi and Cramer's V statistics used at α= 0.05 used.

7.4.2 Infant body composition at 6months of age

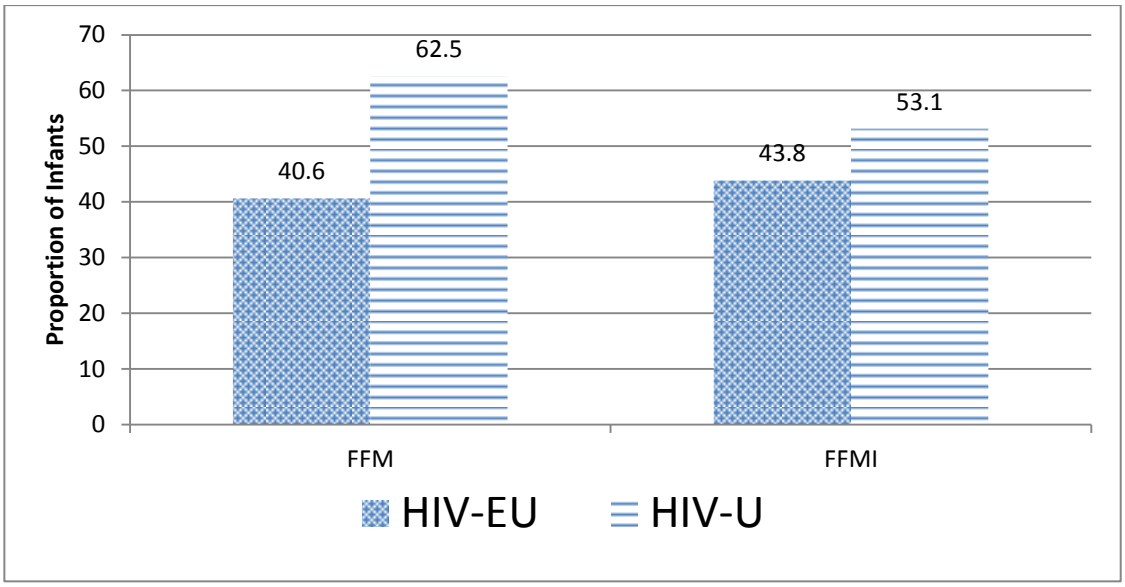
The body composition measurements of the infants using deuterium dilution method at 6 months of age are shown in Table 6.3. Lean mass (5.7 for HIV-EU and 5.9 for HIV-U), fat mass (1.6 for HIV-EU and HIV-U) and %fat mass (22.3% for HIV-EU and 21.3% for HIV-U) were comparable between the two groups (p>0.05). The two body composition indices, fat free mass index and fat mass index were computed by dividing the two by the square of the infant height. These also compared favorably among the two groups of infants (p=0.073 and 0.35 for fat free mass index and fat mass index respectively (Table 7.3).

Table 7.3: Infant body composition at 6 months of age

Variables	HIV-EU (n=32)	HIV-U (n=32)	P-value[∞]
Fat free mass (Kg)	5.7 ±0.7	5.9 ±0.7	0.10
Fat mass (Kg)	1.6 ±0.4	1.6 ±0.6	1.00
% fat mass	22.3 ±4.4	21.3 ±5.7	0.34
Fat free mass index (kgm^{-2})	14.7 ±2.3	14.5 ±1.3	0.73
Fat mass index (kgm^{-2})	4.3 ±1.3	4.0 ±1.3	0.35

[∞]P-value based on students t-test for independent samples at α= 0.05.

Adjusted for maternal height, maternal % fat mass, maternal education, maternal age, infant sex, and infant birth weight, length-for-age difference between 6 weeks and 6 month and breast milk intake, there were no odds for any of the two groups of infants having a greater proportion of infants above their median fat free mass and fat free mass index (Figure 7.1).



[∞]Adjusted Odds Ratio for FFM is 0.915; 95% CI 0.136-6.143; p-value 0.93 and for FFMI is 0.323; 95% CI 0.065-1.604; p-value 0.167. Odd ratio adjusted for the maternal height, maternal %FM, maternal education, maternal age, infant sex, infant birth weight, breast milk intake and LAZ difference between 6 weeks and 6 months.

Figure 7.1: Proportion of infants above the median FFM and FFMI

Among the infants born of HIV-positive mothers body composition variables were compared by maternal ART intake and CD4 cell count (Table 7.4). Infant

fat free mass, % fat mass and the fat free mass index differed between those on ART and non-ART. Infants of non-ART mothers had significantly higher lean mass (6.0 kg) than infants of mothers on ART (5.4 kg), and there was significant differences. Corresponding % fat mass was lower in non-ART as compared to ART counterparts. Lean mass did not differ with the stage of HIV progression as indicated by the maternal CD4 cell count. Fat mass index was however higher among infants of mother with CD4 cell count of <350cells/mm^3 ($p \leq 0.05$).

Table 7.4: Body composition of infants among HIV-exposed infants by maternal ART and CD4 cell count

Variables	ART status		CD4 cell count	
	ART (n=22)	Non-ART (n=10)	CD4<350cells/ mm^3 (n=5)	CD4≥350 cells/ mm^3 (n=16)
Fat free mass (Kg)	5.4*±0.5	6.0*±0.9	5.6±0.6	5.6±0.8
Fat mass (Kg)	1.7±0.3	1.4±0.4	1.9±0.3	1.5±0.3
% fat mass	24.0*±3.6	19.3*±4.5	25.0±4.2	21.3±3.2
Fat free mass index (kgm^{-2})	14.2*±1.9	16.0*±2.8	14.3±1.5	14.3±1.6
Fat mass index (kgm^{-2})	4.5±1.2	3.8±0.9	4.8*±1.0	3.9*±.7

*Values with p-values <0.05 based on students t-test for independent samples at $\alpha = 0.05$.

7.4.3 Correlates of body composition of 6 months old infants

Fat free mass, fat free mass index, fat mass and fat mass index was correlated with maternal and infant variables collected (Table 7.5). Statistical significances were tested at both 0.01 and 0.05 levels. Maternal variables positively correlated with body composition were BMI (r=0.402 for fat free mass), % maternal fat mass (r= 0.402 for .253 for fat mass) and ART status (r=0.415, 0.316, 0.350 for fat free mass, fat free mass index and fat index). Breast milk intake at 6 weeks post-partum was positively correlated with lean mass and FFM index (r=0.227 and 0.28 respectively). Breast milk intake at 6 months were also positively correlated with lean mass, fat free mass index and fat mass (r=0.655, 0.246, 0.252 and 0.275 respectively). Exclusive breastfeeding practice did not depict significant correlation with body composition variables.

Table 7.5: Maternal and infant correlates of body composition at 6 months of age of infants

	Coefficient of correlations (r)			
	Fat free mass (lean mass)	**Fat free mass index**	**Fat mass**	**Fat mass index**
Maternal variables				
HIV status	0.210	-0.045	0.000	-0.120
Age	-0.015	0.042	0.205	0.246
Body Mass Index	0.380**	0.205	0.093	-0.002
Lean mass	0.102	-0.074	0.051	-0.025
% Fat Mass	0.402**	0.109	0.253*	0.091
Height	0.058	-0.060	-0.020	-0.077
ART status∞	0.415*	0.361*	0.350*	0.318
CD4 cell count∞	0.113	0.118	-0.247	-0.256
Infant variables				
Sex	-0.169	-0.033	-0.281*	-0.243
Birth weight	0.050	0.126	-0.138	-0.125
Birth order	0.120	0.179	0.017	0.041
WAZ score at 6 weeks	0.233	-0.334**	0.077	-0.193
Breast milk intake (6 weeks) ¥	0.227*	0.281*	0.155	0.192
Breast milk intake at 6 months ¥	0.645**	0.246*	0.252**	0.043
Change in breast milk intake	0.265*	-0.047	0.064	-0.129
Change in non-breast milk water intake	-0.140	-0.027	0.069	0.085
EBF at 6 wk¥	0.128	0.204	-0.014	-0.287
EBF at 6mo	0.118	0.103	0.091	0.103

∞Only for HIV-1 positive mothers where n=32 for ART analysis and n=21 at 6 months for CD4 cell count analysis

¥As determined by deuterium oxide dose-to-mother technique. Change is between 6 weeks and 6 months values

*Pearsons correlation is significant at the 0.05 level ($p<0.05$)

**Pearsons correlation is significant at the 0.01 level ($p<0.01$)

7.4.4 Prediction equations for body composition for HIV-Uninfected infants

Three equations (Bandana *et al*., 2010; Shailk and Dulip, 2004 Liu *et al*., 2010) formulated for predicting % fat mass of infants were validated against deuterium dilution method for use among 6 months old Kenyan infants. These equations use

infant skin fold thicknesses, MUAC and age. When used on study infants, they yielded varying values of % fat mass (Table 7.6). Bandana *et al*. (2010) gave higher values for both males and females (31.7%, and 21.1%) compared to other equations while equation by Liu *et al.* 2010 yielded relatively lower figures (14.2% for males and 17.5% for females. Compared to the % fat figures as determined by deuterium oxide dilution technique (Table 7.3), it appeared that Bandana *et al*. (2010) overestimated and the Liu *et al*. (2010) underestimated the % fat mass.

Table 7.6: Percent fat mass of 6 months old Kenyan HIV-uninfected using prediction equations

Predictor equations	**% Fat Mass**	
	Males (n=27)	**Females (n=38)**
Bandana *et al*., 2010	31.7 ± 7.7^{b}	21.1 ± 7.9^{b}
Shailk and Dulip, 2004	29.5 ± 2.1^{b}	18.8 ± 1.0^{c}
Liu *et al*., 2010	14.2 ± 2.1^{a}	17.5 ± 2.2^{a}

Different superscript in a column denotes no statistical significance of the indicated differences. Statistical differences are computed by Duncan's post hoc Analysis of variance (ANOVA).

Pairwise comparisons for males and females separately for the predicted verses the deuterium dilution method determined % fat mass are depicted in Table 7.7. The average of the two % fat mass (predicted verses deuterium dilution determined) and the differences are compared. None of the equations depicted no bias between the predicted % fat mass and the deuterium dilution determined (bias column). Liu *et al*. (2010) equation showed the highest bias among the males as well as among the females. Shailk and Dulip (2004) equation yielded the lowest biase among the males while Bandana *et al*. (2010) among the females. The slope and the intercept represented the regression equations between the average and the differences in % fat mass while the p value indicated if there was a relationship (or) not. There was significant relationship between the differences and average in all the equations under validation ($p<0.05$) for both males and females. This indicated that the differences would not remain constant and would change depending on the value of the % fat mass. Also computed were the bias -2SD and

+SD shown in the last two columns which showed the possible lower and higher SD respectively.

Table 7.7: Bland and Altman pair-wise comparison between the prediction equations and deuterium dilution values of infant Fat Mass

	Bias	SD	Slope	Intercept	P	Bias - 2SD	Bias +SD
Males							
Bandana *et al*., 2010	8.4	8.9	0.50	-19.9	0.007	5.1	11.8
Shailk and Dulip, 2004	6.2	5.1	-0.67	41.6	0.001	4.3	7.0
Liu *et al*., 2010	-9.0	4.9	-0.66	15.3	0.000	3.0	6.7
Female							
Bandana *et al*., 2010	0.11	8.3	0.29	-12.0	0.026	5.6	11.0
Shailk and Dulip, 2004	-2.2	5.0	0.81	29.9	0.000	3.4	6.7
Liu *et al*., 2010	8.2	5.2	0.60	12.1	0.000	3.6	7.0

7.5 Discussions

The present study revealed that 6 months aged HIV-negative infants maternally exposed to HIV did not have different body composition compared to the HIV-unexposed infants. Infants born of positive mothers on ART accumulated less lean mass than those not on ART. Amount of breast milk intake earlier in the life (at 6 weeks and 6 months) was positively and significantly correlated with lean mass of HIV-uninfected infants at 6 months of age. None of the currently existing predictor equations for % fat mass in infants of 6 months old (Bandana *et al*., 2010; Shailk and Dulip, 2004 Liu *et al*., 2010) reliably yield comparative figures to those generated by deuterium dilution method.

The % fat mass figures found in the present study were slightly lower by ~5% as compared to those reported elsewhere for the same age group (Fomon *et al*., 1982; Field *et al*., 2011). The similarity in some maternal and infant characteristics (between HIV-EU and HIV-U) may explain the corresponding similarity in body composition. Infant lean body mass is associated with maternal height (Harvey *et*

al., 2005) and maternal BMI (Hull *et al*., 2008). Associated with lean mass are also birth weight (Wells *et al*., 2007) and gestation age (Lapillonne *et al*., 2008) and both were comparable between the two groups. Breast milk intake which is positively correlated with lean mass was also similar between the two groups (chapter 4). With current HIV treatment regimen, negative birth outcomes among HIV-positive mothers are averted (Schulte *et al*., 2007) and thus limited differences between infant born of HIV-positive mothers and those born of HIV-negative mothers.

Among the HIV-positive mothers, use of ART seemed to decrease lean mass and corresponding increase fat mass. ART are passed to infant through the placenta (intrauterine) (Afran *et al*., 2004). In addition, HIV-1 inhibitory concentrations of nevirapine are achieved in breast-feeding infants of mothers receiving these ARVs, exposing infants to the potential for beneficial and adverse effects of nevirapine ingestion (Shapiro *et al.,* 2005). Among the HIV infected infants provision ART have been known to alter fat lipodystrophy (Arpadi, 2000) which include distribution of adipose tissue. The influence of ART on body composition of HIV-infected infant on ART has been reported (Kim *et al.,* 2010; Arpadi, 2013). It is perhaps possible that pharmacological or non-pharmacological amounts of ART passed to the infants may have a reducing effect of lean mass. In this study location, mothers are routinely tested of HIV when pregnant and put on ART (Tenofovir- Effavirenz combination or Tenofovir- Effavirenz- nevirapine combination) and antibiotics (septrin).

Amount of breast milk intake earlier in life (at 6 weeks and 6 months) was positively correlated with lean mass – the higher the infant intake of breast milk was correlated with higher infant lean mass. It is therefore reasonable that the similarity in amount of breast milk consumed by the two groups at the two points of measurements (as depicted in chapter 4), could also explain their compared body composition. There are no indications that exclusive breastfeeding practice is associated with lean mass early in life of HIV-uninfected infant. This is consistent with the review by Gianni *et al.* (2014) indicating that given the

available studies by then, it was still inconclusive that feeding practices influenced body composition.

The three prediction equations using skin fold thicknesses do not yield comparable % fat mass values and have significant biases with deuterium dilution. This may be due to the area specificity of the equations. For instance, the Bandana *et al*. (2010) equation which yielded highest values of % fat mass was designed for Indian 6-24 months old. Bandara *et al.* (2014) also used this equation and found relatively higher values of % fat mass as compared to other equations that they tested for Sri Lankan infants. In this present study, Bandana *et al.* (2010) equation yielded relatively very small biase of 0.11 among the female but the p was <0.05, indicating the differences would not remain constant and would depend on the % fat mass. The relatively small biase could also be explained by the body composition method used for developing the equation. Bandana *et al.* (2010) used the deuterium dilution method, just as the present study. The rejection of use of the validated equations calls for formulation of Kenyan specific body composition prediction equations for % fat mass.

7.6 Conclusions and Recommendations

Although in overall infants of HIV positive mothers have similar lean mass to that of HIV-negative mother by 6 months after birth, ART passed from the mother to infant through breast milk may promotes infant adiposity. No equation reliably predicts the deuterium dilution determined body composition for Kenyan infants, necessitating the formulation of Kenya-specific body composition prediction equations.

7.7 References

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CHAPTER EIGHT

8 GENERAL DISCUSSION, CONCLUSIONS AND RECOMENDATIONS

8.1 General Discussion

Currently, the breastfeeding recommendation for all infants irrespective of maternal HIV status is to be breastfed exclusively for the first 6 months of their lives and there after appropriate complementary feeding introduces as breastfeeding is continued to two years and beyond (WHO, 2010). With this recommendation in place, this study assessed if there are any differences in breastfeeding practices, breast milk intake, growth and body composition among the HIV-exposed, uninfected and HIV-unexposed infants. It was found that a greater proportion of the HIV-positive mothers exclusively breastfed as compared to the HIV-negative mothers by 6 months of age. This may have been due to a greater exposure to counseling on recommended breastfeeding practices by the HIV-positive mothers. The HIV-positive mothers are also presumably fearful of the risk of virus transmission to the infant which comes with mixed feeding practice. This study has revealed using dose-to-mother deuterium oxide (DO) technique that at 6 weeks postpartum, 23% HIV-EU infants adhere to the WHO 2010 guideline on exclusive breastfeeding while 43% do at 6 months. These values were lower than those recalled by mothers, and this study provides possible correction factors for estimating actual rate after mothers recall. The cost of DO technique is however high. Consequently, an innovation that would lead to cheaper way to measure deuterium enrichment will make a significant contribution to the measurement of EBF.

There is no difference in breast milk intake between infants born of HIV-positive mothers and those born of HIV-negative mothers and the two groups consumed roughly normative amounts expected at 6 weeks postpartum and 6 months of age as reported by da Costa *et al.,* (2010). There is also a possibility of a compensatory effect where the likelihood of low breast output among HIV-positive mothers is compensated for by their better breastfeeding practices. Most mothers (~60%) were on combination ART (Tenofovir- Effavirenz combination

or Tenofovir- Effavirenz- nevirapine combination) and majority (~77%) with CD4 cell counts of >350. ART ensures that these HIV-positive mothers are less affected by opportunistic illnesses and thus able to effectively care for and breastfeed their infants. Infections (such as subclinical mastitis) have been shown to affect breast milk output. High levels of breast milk RNA viral load are associated with the occurance of sub-clinical mastitis (Wilumsen *et al.,* 2003). However, ART started during pregnancy or postpartum suppresses breast milk HIV-1 RNA (Shapiro *et al.,* 2005; Chung *et al.,* 2008). This may explain why the observation of reduced breast milk with the occurrence of sub-clinical mastitis in dairy cows (Shuster *et al.,* 1995) is not consistent with the findings of this study.

The amount of breast milk intake was positively associated with growth of HIV-uninfected infants at 6 months postpartum and significantly accounted for the change in growth between 6 weeks and 6 month after birth. Further, infants who consumed more breast milk were more likely to have higher lean mass. It is therefore not surprising that since HIV-EU and HIV-U consumed on average similar amounts of breast milk, their linear growth and weight were also similar by 6 months of age. HIV-EU however had poorer linear growth at 6 weeks postpartum and this may be due to factors including those related to HIV or ART exposure intrauterine. This study did provide the revelation that the body composition for HIV-EU infants is not any different from those of HIV-U. This is explained by the similarity of the two groups in breast milk intake, maternal height, maternal BMI, gestational age and birth weight. These factors are associated with infant body composition (Harvey *et al*., 2005; Hull *et al*., 2008; Lapillonne *et al*., 2008). Unlike growth, lean mass of infants was influenced by maternal ART intake. The results on the link between maternal ART and infant body composition is however only indicative, calling for studies to be specifically designed to confirm the variations. Methodologies for measuring body composition are expensive and the use of available predictive equations (using skin fold thicknesses and anthropometric measurements) provide an option for quick estimation. This present study however found out that none of the equations

developed for infants under 6 months old reliably predict body composition (% fat) as measured by deuterium dilution method of estimating Total Body Water.

The study recruited mother-infant dyads from the health facility set-up. This group is not fully representative of the general population. In Kenya, about half of women receive post-natal care (GOK, 2014). The mothers sampled were thus exposed to nutrition and health counseling and thus the findings of this study may be representative of those with exposure to health facilities and/or health workers.

8.2 Conclusions

Within the exclusive breastfeeding age bracket in resource poor settings, maternal HIV status does not influence the breast milk intake of HIV-uninfected infants. Infants of HIV-positive mothers are however more likely to be exclusively breastfed compared to infants of HIV-negative mothers. Maternal recalls tend to over-estimate exclusive breastfeeding rate when compared to deuterium oxide dilution technique. Interventions that ensure normal maternal body mass index, maternal lean mass, infant size (birth and current weight) are important in increasing breast milk intake which is in turn important in promoting growth and lean mass of HIV-uninfected infants.

8.3 Recommendations

For policy and practice

1. Validation of self-reported EBF practices with the low-cost, non-invasive deuterium oxide dilution technique is highly recommended to facilitate more effective breastfeeding promotion campaigns.
2. Interventions that ensure normal maternal body mass index, maternal lean mass, infant size (birth and current weight) should be scaled-up to increase breast milk output and for better infant growth and body composition.
3. Re-enforce antenatal and postnatal counselling for mothers regardless of HIV-status. Study found higher exposure to counselling by HIV-positive mothers and higher EBF rates among this group.

For further research

4. The finding that infants of mothers on ART showed lower lean mass may need further investigation with a specific study designed to detect the variations.
5. There is need for formulating Kenya-specific % fat prediction equations for infants. This will lead to a better understanding of the body composition of Kenyan infants in a wider scale.

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ANNEXES

ANNEX 1: CONSENT FORM

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF NAIROBI INSITUTE OF TROPICAL AND INFECTIOUS DISEASES (UNITID)

Consent for participants in a study titled

Effects of maternal HIV status on breast milk intake and growth of Kenyan HIV-uninfected infants at 6 weeks post-partum and 6 months of age

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Hello, my name is _____. I work with the University of Nairobi Institute of Tropical and Infectious Disease (UNITID).

Introduction
University of Nairobi, College of Health Science is conducting a research to determine breast milk intake and growth of children who are HIV-negative, both born of HIV-positive and negative mothers. This study will measure breast milk intake and growth of a child when it is 6 weeks and 6 months old after birth. The aim of the study is to see if there are differences between children who are HIV-exposed but are not infected and those who are not exposed and not infected on terms of milk intake and growth. The study will provide valuable data to inform policy and programming that will improve care for children born of HIV-positive mothers but are not infected.

This study will take 6-7 months after you have given birth but the interviewing and measurements will be done when the child is 6 weeks and 6 months old. For this reason, we are only interested with participants who will stay in their current locations, and will not relocate in the next 8 months.

HIV tests for the mother and baby

As we have informed, we are only interested with children who are HIV-negative, but mothers who are either HIV-positive or negative. To know your HIV status, we will allow you to have routine HIV test at Siaya District Hospital after you agree to be tested. If you have already been tested, we will request you to give us permission to have access to your results from the medical records. When the child is born, the baby will be tested of HIV before 6 weeks. Only children who are HIV-negative will be recruited for the study, irrespective of the mothers HIV status.

Questionnaires
After 6 weeks after birth and when the child is 6 months old, you will be asked questions about family situation, your health, child's health and development and

how you are breastfeeding the child. In some cases, you be asked for your clinic card and drug prescriptions. You will also be asked questions on your child development, and the child's weight, height, arm and head will be measured. We also ask you the questions related to food access in your household. You will not have to answer any questions that you do not want to answer.

Breast milk intake

When the baby is 6 weeks and 6 months old, we will measure the child body size by giving the mother the staple isotope deuterium-labelled water (special water). The staple isotope deuterium-labeled water is naturally occurring, non-radioactive and no health risk at all. Deuterium does already exist naturally in your body fluid. First we will sample saliva from you and your child using cotton wool and syringe. We will then give you a small amount (30g) of special water to drink. The following day, almost at the same time you will have been given the special water, your saliva and the baby's will again be collected. This will be called day 1 of the collection. This will be repeated in day 2, 3, 4, 13 and 14.

Body composition

At the beginning and the end of the study, we will measure the child body size by staple isotope deuterium-labelled water. First we will sample saliva from your child, about one teaspoon (max. 2 ml), then give your child staple isotope deuterium-labelled water ($2H_2O$) which is diluted in normal drinking sterile water (about one spoon = 10 mL). We need you to wait at the mobile-clinic for three hours, where we again will sample one teaspoon saliva from your child. The staple isotope deuterium-labeled water is naturally occurring, non-radioactive and no health risk at all. Deuterium does already exist naturally in your body fluid.

Study participant (mother) consent

I have been fully informed of this study and I am aware that should I not wish to participate in this study it will not affect the treatment of myself or my child. Equally should I consent to participation I will not be given any special services or be given payment or gifts.

I agree to allow a visit to my home for interview and that I will allow the study group to ask me questions regarding the living situation of my family and health status of me and my child.

I agree to allow the special water to be given to me and my child to drink when provided on selected days when my child is 6 weeks and 6 months old.

I agree that I will allow the study group to collect saliva from myself and my child over the selected days.

I agree to allow the study group to measure my child's weight, length, head circumference and skinfolds

I agree to allow the study group to measure my weight and height.

This consent is only valid for this study. I hereby consent to participate.

Do you have any questions? If at any time during the study you have any questions, you can contact: The researcher whose contacts are given below:

Shadrack Oiye
Institute of Tropical and Infectious Diseases, University of Nairobi
P.O. Box 19676-00202, NAIROBI.
Mobile: 0722-759449
Email: oiyeshad@gmail.com

We would like to ask for your participation in the study now. If you agree to participate with your child in the study, please sign or mark in the box below.

Signature or thumbprint of the caregiver: Date
_____/_____/_____

For study fieldworker
I have read the consent form in its entirety to the caregiver of the child.

Signature of study fieldworker:_____

Name (in CAPITAL LETTERS)

Date (day/month/year): _____/_____/_____

ANNEX 2: STUDY TOOLS

Annex 2.1: 6 Weeks Post-Partum Questionnaire

SIAYA BREASTMILK INTAKE STUDY

SIX WEEKS POSTPARTUM QUESTIONNAIRE

INSTRUCTIONS TO THE INTERVIWER

1. Do not conduct the interview if the mother did not consent
2. Do not read responses unless instructed to do so
3. On top of each page, write the mother's ID

INSTRUCTION TO THE MOTHER

We are going to spend about 45 minutes to 1 hour asking you questions about your household, yourself and your child. You are expected to provide accurate answers. If you are not comfortable in providing response, you are free not to provide it.

We are now going to ask you questions about yourself, household and infant.

IDENTIFICATION INFORMATION (Interview the mother)
INTERVIEW DATE (DD/MM/YYYY)|__|__|/|__|__|/|__|__|__|__|
INTERVIEWER NAME/ID _____/|__|__|__|__|
CHILD ID: |__|__|__| __| Name: _____
DATE OF BIRTH OF CHILD (DD/MM/YYYY)|__|__|/|__|__|/|__|__|__|__|
CHILD SEX (1=MALE, 2=FEMALE): |__|
MOTHER ID |__|__|__|__|Name: _____
OTHER NAMES USED BY THE MOTHER
DATE OF BIRTH OF MOTHER (DD/MM/YYYY)|__|__|/|__|__|/|__|__|__|__|
MOTHER TELEPHONE NUMBER |__|__||__||__|/|__|__||__|__|__|__|
DISTRICT ID: |__|__|__| Name: _____
LOCATION ID: |__|__|__| Name:

_____		
SUBLOCATION ID: |__|__|__| Name: _____		
VILLAGE ID: |__|__|__| Name: _____		
CLOSSEST SHOPPING CENTRE TO THE VILLAGE/HOME		
NAME OF HOUSEHOLD HEAD _____ HOUSEHOLD HEAD TELEPHONE NUMBER |__|__||__||__|/|__|__||__|__|__|__|		
NAME OF CHW _____ TELEPHONE NUMBER OF CHW |__|__||__||__|/|__|__||__|__|__|__|		
GPS COORDINATES OF THE RESIDENCE OF MOTHER AND INFANT N |__|__|.|__|__|__|__||__|° E|__||__|__|.|__|__|__|__||__|°		
1.0 DEMOGRAPHIC AND SOCIOECONOMIC STATUS **(Administered to the mother)**		
1.1	Where do you **MOSTLY** stay?	[1] IN THE TOWN (SIAYA TOWN) [2] VILLAGE [3] OTHER (SPECIFY) _____
1.2	Where was your place of birth for the last child (the child that you have now)?	[1] HOME [2] CLINIC [3] HOSPITAL [4] OTHER (SPECIFY) _____
1.3	What was the type of delivery for the last child that you have?	[1] NORMAL DELIVERY [2] CEASARIAN SECTION [3] OTHER (SPECIFY)

		_____
1.4	How many pregnancies have you heard so far?	|__|__|
1.5	How many live children have you given birth to?	|__|__|
1.6	How many living children do you have now?	|__|__|
1.7	Number of own children who are under five years old	|__|
1.8	Gestational age of the last child **(CHECK THE CLINIC CARD)** **WHEN THERE IS NO GESTATIONAL AGE RECORD, INSERT 99.99**	|__|__| MONTHS
1.9	Weight of the infant at birth **(CHECK THE CLINIC CARD)** **WHEN THERE IS NO BIRTH WEIGHT RECORD, INSERT 99.99**	|__|__|.|__|__| KILOGRAMS
1.10	Marital status **IF NOT MARIED, GO TO QUESTION 1.13**	[1] SINGLE [2] MARRIED [3] DIVORCED [4] SEPERATED [5] OTHER (SPECIFY)_____ __
1.11	**IF MARRIED**, are you in an monogamous or polygamous marriage?	[1] MONOGAMOUS [2] POLYGAMOUS [3] OTHER (SPECIFY)_____ __
1.12	Do you live with your spouse	1] YES [2] NO [1] NOT MARRIED
1.13	Which is your religion?	[1] CATHOLIC [2] PROTESTANT [3] MUSLIM [4] OTHER (SPECIFY) _____

1.14	**HIGHEST LEVEL** of education completed by you	[1] NONE [2] NUSERRY/KINDERGARDEN [3] PRIMARY SCHOOL [4] SECONDARY SCHOOL [5] MIDDLE-LEVEL COLLEGE [6] UNIVERSITY [7] ADULT EDUCATION [8] OTHER (SPECIFY)_____ ____
1.15	**HIGHEST** level of education completed by the spouse **ASK ONLY IF MARRIED**	[1] NONE [2] NUSERRY/KINDERGARDEN [3] PRIMARY SCHOOL [4] SECONDARY SCHOOL [5] MIDDLE-LEVEL COLLEGE [6] UNIVERSITY [7] ADULT EDUCATION [8] OTHER (SPECIFY)_____ ____
1.16	Type of house living in	[1] GRASS THATCHED MUD WALL [2] CORRUGATED IRON ROOF MUD WALL [3] CORRUGATED IRON ROOF BLOCK/BRICK WALLS [4] OTHER (SPECIFY)_____ ____
1.17	Does any of the household members own one of the following? **READ RESPONSES. MULTIPLE RESPONSES POSIBLE**	[1] LIVESTOCK [2] BICYCLE [3] MOTOCYCLE/SCOOTER [4] ANIMAL DRAWN CART [5] CAR/ TRUCK [6] TRACTOR [7] WALL CLOCK/WATCH

		[8] RADIO/RADIO CASSET [9] TELEVISION [10] REFGRIGERATOR [11] WATER PUMP
1.18	What is the main source of lighting for the household at night?	[1] ELECTRICITY [2] SOLAR [3] KEROSENE [4] GAS [5] OTHER (SPECIFY)_____ ___
1.19	What type of fuel does the household **MOSTLY** use for cooking?	[1] WOOD [2] CROP RESIDUES [3] DUNG CAKES [4] CHARCOAL [5] KEROSENE [6] ELECTRICITY [7] GAS [8] BIO-GAS [9] OTHER (SPECIFY)_____ _
1.20	Does any of the household members own land?	1] YES [2] NO
1.21	If yes, how many acres of land (altogether) are owned by the members of this household?	|__||__||__|.|__| ACRES
1.22	What is your main economic activity?	[1] HOUSEWIFE [2] EMPLOYED [3] SELF EMPLOYED (BUSSINESS) [4] FARMER [4] OTHER (SPECIFY)_____ ___
1.23	Which source do you **MOSTLY** get water for drinking from?	[1] BOREHOLE [2] UNPROTECTED SPRING [3] PROTECTED SPRING

		[4] UNPROTECTED DUG WELL [5] PROTECTED DUG WELL [6] RAINWATER COLLECTION [7] CART WITH SMALL TANK/DRUM [8] TANKER TRUCK [9] SURFACE WATER (RIVER, DAM, LAKE, POND, STREAM, ETC.) [10] PIPED WATER INTO DWELLING [11] PIPED WATER INTO YARD/PLOT [12] OTHER (SPECIFY):_____
1.24	Where do you **MOSTLY** defecate (go to the toilet) **KWA KAWAIDA**, munaendea choo wapi?	[1] FLUSH/POUR FLUSH [2] VENTILATED IMPROVED PIT LATRINE (VIP) [3] PIT LATRINE WITH SLAB [4] PIT LATRINE WITHOUT SLAB/OPEN PIT [5] COMPOSTING TOILET [6] BUCKET HANGING TOILET/HANGING LATRINE [7] IN THE BUSH [8] IN THE OPEN SPACE OUTSIDE THE HOUSE [9] OTHER (SPECIFY):_____
2.0 BREAST HEALTH AND CARE **Now I will ask you questions about the health of your breast.**		
2.1	For your previous child other than the current one [NAME OF INFANT] , did you breastfeed? **IF YES, GO TO QUESTION 2.3**	1] YES [2] NO **[3] NOT APPLICABLE (THE CHILD IS THE FIRST BORN)**
2.2	**IF NO** above, state the reason	[1] WAS ADVISED NOT TO BREASTFEED BY HEALTH WORKER [2] WAS ADVISED NOT TO BREASTFEED BY FRIENDS/RELATIVE

		[3] I WAS SICK [4] CHILD WAS SICK [5] PAIN DURING BREASTFEEDING [6] INVERTED NIPPLES [7] OTHER (SPECIFY) _____
2.3	**IF YES,** for how many months did you breastfeed?	|__||__|
2.4	Have you been given advice on how to take care of your breast? **IF NO, GO TO QUESTION 2.6**	1] YES [2] NO
2.5	If **YES** above, which advice?	_____ _____ _____ _____ _____
2.6	What problems are you currently experiencing with any of your breasts? **[MULTIPLE RESPONSES POSIBLE]**	[1] NO PROBLEM [2] PAIN DURING BREASTFEEDING [3] INVERTED NIPPLES [4] BLOCKED NIPPLES OF ONE OF THE BREAST [5] OTHER (SPECIFY) _____
3.0 BREASTFEEDING PRACTICES **Now I will ask you questions about the breast feeding practices**		
3.1	How long after birth did you first put **[NAME OF INFANT]** to the breast after birth?	[1] IMMIDIATELY [2] IN LESS THAN ONE HOUR [3] AFTER SEVERAL HOURS [4] AFTER A DAY OR MORE [99] DON'T KNOW
3.2	In the first three days of after deliver, was **[NAME OF INFANT]** given anything to drink other than breast milk? **IF NO GO TO QUESTIONS 3.4**	[1] YES [2] NO [99] DON'T KNOW
3.3	**IF YES**, what was the **[NAME OF INFANT]** given?	[1] MILK (OTHER THAN BREASTMILK) [2] PLAIN WATER [3] SUGAR OR GLUCOSE WATER

	[MULTIPLE RESPONSES POSSIBLE]	[4] GRIPE WATER [5] SUGAR-SALT-WATER SOLUTION [6] FRUIT JUICE [7] INFANT FORMULAR [8] TEA INFUSION [9] HONEY [10] OTHER (SPECIFY) _____
3.4	In the last 24 hours, what did you your child? **[MULTIPLE RESPONSES POSSIBLE]**	[1] BREAST MILK [2] PLAIN WATER [3] SUGAR OR GLUCOSE WATER [4] GRIPE WATER [5] SUGAR-SALT-WATER SOLUTION [6] FRUIT JUICE [7] INFANT FORMULAR [8] TEA INFUSION [9] HONEY [10] MILK [11] PORRIDGE [12] HONEY [13] OTHER (SPECIFY)_____
3.5	How many times did you breastfeed last night between sunset and sunrise? **[IF ANSWER IS NOT NUMERIC, PROBE FOR APPROXIMATE NUMBER]**	|__||__| TIMES [99] MANY TIMES [999] CANNOT REMEMBER
3.6	How many times did you breastfeed yesterday during daylight hours **[IF ANSWER IS NOT NUMERIC, PROBE FOR APPROXIMATE NUMBER]**	|__||__| TIMES [99] MANY TIMES [999] CANNOT REMEMBER
3.7	Do you express breast milk before giving it to [NAME OF INFANT]?	[1] YES [2] NO [99] DON'T KNOW
3.8	During pregnancy and after birth, were you given any information or counseling about breastfeeding? ?	[1] YES [2] NO [99] CANT REMEMBER
3.9	Who mostly takes care of [INFANT NAME]]	[1] MOTHER ONLY [2] MOTHER + GRANDMOTHER OF CHILD [3] MOTHER + ADULT FEMALE RELATIVE

		[4] MOTHER + OLDER SIBLING [5] MOTHER + FATHER [6] OTHER (SPECIFY] _____
3.10	Approximately in how many days in a week do you leave [INFANT NAME] attended to by others **IF NEVER LEFT ALONE, INSERT ZERO** **IF THE RESPONSE IS ZERO, GO TO QUESTION 3.13**	INSERT NUMBER **[ ]**
3.11	Who do you MOSTLY leave the [INFANT NAME] with when not at home	[1] MOTHER [2] GRANDMOTHER [3] OTHER ADULT FEMALE RELATIVE [4] SIBLING [5] HIRED CAREGIVER [6] CARE GIVER WHO IS RELATED TO FAMILY OTHER (SPECIFY) _____
3.12	Do you know of any cultural belief on breastfeeding which is related to HIV? **[IF NO OR DON'T KNOW, GO TO SECTION 4]**	[1] YES [2] NO [99] DON'T KNOW
3.13	LIST THE BELIEFS 1. _____ _____ _____ _____ 2. _____ _____ _____ _____ 3. _____ _____	

4.0 MATERNAL MORBIDITY AND MEDICATIONS **Now I will ask you questions concerning your general health**			
	In the last two week have you experienced any of the following illness?		
4.1	Fever	[1] YES	[2] NO
4.2	Cough	[1] YES	[2] NO
4.3	Diarrhea	[1] YES	[2] NO
4.4	Vomiting	[1] YES	[2] NO
4.5	Fits	[1] YES	[2] NO
4.6	Stomachache	[1] YES	[2] NO
4.7	Skin rash	[1] YES	[2] NO
4.8	Difficulty breathing	[1] YES	[2] NO
4.9	Loss of appetite	[1] YES	[2] NO
4.10	Pain in one breast	[1] YES	[2] NO
4.11	Pain in both breasts	[1] YES	[2] NO
4.12	Other, specify	_____ _____ _____	
4.13	Is there any specific disease that you have been diagnosed of in the last one month in the hospital? **IF NO, GO TO QUESTION 4.15**	[1] YES	[2] NO
4.14	If YES above which one	_____	

		_____
4.15	Are you under any kind of medication (s) currently? **IF NO GO TO QUESTION 4.17**	[1] YES [2] NO
4.16	If YES above which ones **[CONFIRM FROM CLINIC CARDS/PRESCRIPTIONS GIVEN]**	_____ _____ _____ _____ _____ _____
4.17	Are you being provided with any form of food supplements for your own consumption (eg. given in the hospital)? **IF NO, GO TO QUESTION 4.1**	[1] YES [2] NO
4.18	**IF YES,** which type of food are you given?	_____ _____
	In the last one week, in how many days have you experienced the following?	WRITE NUMBER (0-7). IF NOT EXPEREINCED, INSERT ZERO
4.19	Feeling of sadness	[ ]
4.20	Loss of interest in your duties	[ ]
4.21	Loss of appetite	[ ]
4.22	Loss of sleep (sleeping problems)	[ ]
4.23	Loss of thinking/concentration	[ ]
4.24	Feeling of guilt (or worthlessness)	[ ]
4.25	Feeling of excessive fatigue	[ ]
4.26	Difficulty in movement	[ ]
4.27	Suicidal feeling	[ ]
5.0 INFANT MORBIDITY AND MEDICATIONS **Now I will ask you questions concerning the health of [NAME OF INFANT]**		

	In the last two week have [NAME OF INFANT] experienced any of the following illness?		
5.1	FeveR	[1] YES	[2] NO
5.2	Cough	[1] YES	[2] NO
5.3	Diarrhea	[1] YES	[2] NO
5.4	Vomiting	[1] YES	[2] NO
5.5	Fits	[1] YES	[2] NO
5.6	Stomachache	[1] YES	[2] NO
5.7	Skin rash	[1] YES	[2] NO
5.8	Difficulty breathing	[1] YES	[2] NO
5.9	Loss of appetite	[1] YES	[2] NO
5.10	Other, specify		
5.11	Is there any specific disease **[NAME OF INFANT]** has been diagnosed of in the last one month in the hospital? **IF NO, GO TO SECTION 6**	[1] YES	[2] NO
5.12	**IF YES** above which one		
5.13	Is **[NAME OF INFANT]** under any kind of medication (s) currently? **IF NO, GO TO SECTION 6**	[1] YES	[2] NO
5.14	If YES above which ones **[CONFIRM FORM CLINIC CARDS/PRESCRIPTIONS GIVEN]**	_____ _____ _____ _____ _____ _____	
6.0 SEASONALITY **Now I will ask you questions concerning seasons and food in your household**			

6.1 What is agricultural curent season	(1) Pre-harvest (2) Harvest (3) In between harvest and pre-harvest (4) Other (specify)

7.0 HOUSEHOLD FOOD INSECURITY AND ACCESS SCALE

FOR EACH QUESTION, IF THE RESPONSE IS "2 = NO" THEN PROCEED TO THE NEXT QUESTION. OTHERWISE ASK THE QUESTION IN THE RIGHT COLLUMN

	Occurrence Questions **READ RESPONSES FOR THE INTERVIEWEE.**	**Response**	**If Yes, how often did it occur?** **1 = Rarely** (once or twice in the past four weeks) **2 = Sometimes** (three to ten times in the past four weeks) **3 = Often** (more than ten times in the past four weeks)
7.1	In the past four weeks, did you worry that your household would not have enough food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.2	In the past four weeks, were you or any household member not able to eat the kinds of foods you preferred because of a lack of resources? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.3	In the past four weeks, did you or any household member have to eat a limited variety of foods due to a lack of resources? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.4	In the past four weeks, did you or any household member have to eat some foods that you really did not want to eat because of a lack of resources to obtain other types of food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.5	In the past four weeks, did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.6	In the past four weeks, did you or any household member have to eat fewer meals in a	(1) YES (2) NO	(1) Rarely (2) Sometimes

	day because there was not enough food? **READ RESPONSES**		(3) Often
7.7	In the past four weeks, was there ever no food to eat of any kind in your household because of lack of resources to get food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.8	In the past four weeks, did you or any household member go to sleep at night hungry because there was not enough food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.9	In the past four weeks, did you or any household member go a whole day and night without eating anything because there was not enough food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.10	Did you have enough food to eat over the past 4 weeks?		(1) Never (2)Usually not (more days with not enough food) (3) Usually (more days with enough food) (4) Always

8. MOTHER'S DEUTERIUM DOSING AND SALIVA COLLECTION DATA

DAY 0 (To)	
INTERVIEW/DOSING DATE –DAY 0 (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time predose saliva sample taken (24hrs format)	|__|__|:|__|__|
Time deuterium dose given (24hrs format)	|__|__|:|__|__|
Deuterium dose (g)	|__|__|.|__|__||__|__| GRAMS
DAY 1 (T_1)	
DAY 1 DATE (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|

Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 2 (T_2)	
DAY 2 DATE (DD/MM/YYYY)	**|__|__|/|__|__|/|__|__|__|__|**
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY3 (T_3)	
DAY 3 DATE (DD/MM/YYYY)	**|__|__|/|__|__|/|__|__|__|__|**
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 4 (T_4)	
DAY 4 DATE (DD/MM/YYYY)	**|__|__|/|__|__|/|__|__|__|__|**
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 13 (T_{13})	
DAY 13 DATE (DD/MM/YYYY)	**|__|__|/|__|__|/|__|__|__|__|**
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 14 (T_{14})	
DAY 14 DATE (DD/MM/YYYY)	**|__|__|/|__|__|/|__|__|__|__|**
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
ANTHROPOMETRY (for each parameter, two measurements should be taken)	
DAY 0 (To)	

Weight (kg) T_o	|__|__|__|.|__| KG |__||__|__|.|__| KG |__||__|__|.|__| KG
Height/ (cm) T_0	|__|__|__|.|__| CM |__|__|__|.|__| CM |__|__|__|.|__| CM
MUAC (mm) T_o	|__|__| .|__|CM |__|__| .|__|CM |__|__| .|__|CM
DAY 14 (T_{14})	
Weight (kg) T_{14}	|__|__|__|.|__| KG |__||__|__|.|__| KG |__||__|__|.|__| KG
Height/ (cm) T_{14}	|__|__|__|.|__| CM |__|__|__|.|__| CM |__|__|__|.|__| CM
MUAC (mm) T_{14}	|__|__| .|__|CM |__|__| .|__|CM |__|__| .|__|CM

9. INFANT'S DEUTERIUM SALIVA COLLECTION DATA	
DAY 0 (T_0)	
DAY 0 (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time baseline saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 1 (T_1)	
DAY 1 DATE (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 2 (T_2)	
DAY 2 (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 3 (T_3)	

DAY 3 (DD/MM/YYYY)	|___|___|/|___|___|/|___|___|___|___|
Time saliva sample taken (24hrs format)	|___|___|:|___|___|
DAY 4 (T_4)	
DAY 4 (DD/MM/YYYY)	|___|___|/|___|___|/|___|___|___|___|
Time saliva sample taken (24hrs format)	|___|___|:|___|___|
DAY 13 (T_{13})	
DAY 13 (DD/MM/YYYY)	|___|___|/|___|___|/|___|___|___|___|
Time saliva sample taken (24hrs format)	|___|___|:|___|___|
DAY 14 (T_{14})	
DAY 14 (DD/MM/YYYY)	|___|___|/|___|___|/|___|___|___|___|
Time saliva sample taken (24hrs format)	|___|___|:|___|___|
ANTHROPOMETRY (for each parameter, take three measurements)	
DAY 0 (To)	
Weight (kg) T_o	|__|__|. |__|__| |__|KG |__|__|. |__|__|__||KG |__|__|. |__|__|__|KG
Length/ (cm) T_0	|__|__|__|. |__|CM |__|__|__|. |__|CM |__|__|__|. |__| CM
MUAC (mm) T_o	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Head circumference	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Bicept skinfold thickness	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Tricep skinfold thickness	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Subscapular skinfold	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Suprailiac skinfold	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Presence of Bipedal oedema	**[1]** YES [**2]** NO
DAY 14 (T_{14})	
Weight (kg) T_{14}	

	|__|__|• |__||__| |__|KG |__|__|. |__||__|__||KG |__|__|. |__||__||__|KG
lenghth/ (cm) T_{14}	|__|__|__|• |__|CM |__|__|__|• |__|CM |__|__|__|• |__| CM
MUAC (mm) T_{14}	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Head circumference	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Bicept skinfold thickness	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Tricep skinfold thickness	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Subscapular skinfold	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Suprailiac skinfold	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Presence of Bipedal oedema	[1] YES [2] NO
In the last 1 day (24 hours), what food have you given the child **[DO NOT READ RESPONSES]**	[1] BREASTMILK ONLY [2] BREASTMILK AND OTHER SOLID FOODS [3] OTHER FOODS OTHER THAN BREASTMILK [4] OTHER SPECIFY _____

Any observations or comments (report any observations or incidences eg. estimated spillages of deuterium um)

_____
_____
_____
_____
_____
_____

Annex 2.2: 6 Months Age of Infant Questionnaire

SIAYA BREASTMILK INTAKE STUDY

SIX MONTHS QUESTIONNAIRE

INSTRUCTIONS TO THE INTERVIWER

4. Do not conduct the interview if the mother did not consent
5. Do not read responses unless instructed to do so
6. On top of each page, write the mother's ID

INSTRUCTION TO THE MOTHER

We are going to spend about 45 minutes to 1 hour asking you questions about your household, yourself and your child. You are expected to provide accurate answers. If you are not comfortable in providing response, you are free not to provide it.

We are now going to ask you questions about yourself, household and infant.

IDENTIFICATION INFORMATION (Interview the mother)		
INTERVIEW DATE (DD/MM/YYYY) |__|__|/|__|__|/|__|__|__|__|		
INTERVIEWER NAME/ID _____/|__|__|__|__|		
CHILD ID: |__|__|__| __| Name: _____		
CHILD SEX (1=MALE, 2=FEMALE): |__|		
MOTHER ID |__|__|__|__|Name: _____		
8.0 DEMOGRAPHIC AND SOCIOECONOMIC STATUS **(Administered to the mother)**		
1.10	Marital status **IF NOT MARIED, GO TO QUESTION 1.13**	[1] SINGLE [2] MARRIED [3] DIVORCED [4] SEPERATED [5] OTHER (SPECIFY)_____ ___
1.13	Which is your religion?	[1] CATHOLIC [2] PROTESTANT

		[3] MUSLIM [4] OTHER (SPECIFY) _____
1.18	What is the main source of lighting for the household at night?	[1] ELECTRICITY [2] SOLAR [3] KEROSENE [4] GAS [5] OTHER (SPECIFY)_____ ____
9.0 BREAST HEALTH AND CARE Now I will ask you questions about the health of your breast.		
2.4	Have you been given advice on how to take care of your breast in the last 6 months? **IF NO, GO TO QUESTION 2.6**	1] YES [2] NO
2.5	If **YES** above, which advice?	_____ _____ _____ _____ _____
2.6	What problems are you currently experiencing with any of your breasts? **[MULTIPLE RESPONSES POSIBLE]**	[1] NO PROBLEM [2] PAIN DURING BREASTFEEDING [3] INVERTED NIPPLES [4] BLOCKED NIPPLES OF ONE OF THE BREAST [5] OTHER (SPECIFY) _____
10.0 BREASTFEEDING PRACTICES Now I will ask you questions about the breast feeding practices		
3.4	**IF YES**, what was the **[NAME OF INFANT]** given? **[MULTIPLE RESPONSES POSSIBLE]**	[1] MILK (OTHER THAN BREASTMILK) [2] PLAIN WATER [3] SUGAR OR GLUCOSE WATER [4] GRIPE WATER [5] SUGAR-SALT-WATER SOLUTION [6] FRUIT JUICE [7] INFANT FORMULAR [8] TEA INFUSION

		[9] HONEY [10] OTHER (SPECIFY) _____
3.5	How many times did you breastfeed last night between sunset and sunrise? **[IF ANSWER IS NOT NUMERIC, PROBE FOR APPROXIMATE NUMBER]**	|__||__| TIMES [99] MANY TIMES [999] CANNOT REMEMBER
3.6	How many times did you breastfeed yesterday during daylight hours **[IF ANSWER IS NOT NUMERIC, PROBE FOR APPROXIMATE NUMBER]**	|__||__| TIMES [99] MANY TIMES [999] CANNOT REMEMBER
3.7	Do you express breast milk before giving it to [NAME OF INFANT]?	[1] YES [2] NO [99] DON'T KNOW
3.8	During pregnancy and after birth, were you given any information or counseling about breastfeeding? ?	[1] YES [2] NO [99] CANT REMEMBER
3.9	Who mostly takes care of [INFANT NAME]]	[1] MOTHER ONLY [2] MOTHER + GRANDMOTHER OF CHILD [3] MOTHER + ADULT FEMALE RELATIVE [4] MOTHER + OLDER SIBLING [5] MOTHER + FATHER [6] OTHER (SPECIFY] _____
3.10	Approximately in how many days in a week do you leave [INFANT NAME] attended to by others **IF NEVER LEFT ALONE, INSERT ZERO** **IF THE RESPONSE IS ZERO, GO TO QUESTION 3.13**	INSERT NUMBER **[ ]**
3.11	Who do you MOSTLY leave the [INFANT NAME] with when not at home	[1] MOTHER [2] GRANDMOTHER [3] OTHER ADULT FEMALE RELATIVE [4] SIBLING

		[5] HIRED CAREGIVER [6] CARE GIVER WHO IS RELATED TO FAMILY OTHER (SPECIFY) _____
3.12	Do you know of any cultural belief on breastfeeding which is related to HIV? **[IF NO OR DON'T KNOW, GO TO SECTION 4]**	[1] YES [2] NO [99] DON'T KNOW
3.13	LIST THE BELIEFS 4. _____ _____ 5. _____ _____ 6. _____ _____	

11.0 MATERNAL MORBIDITY AND MEDICATIONS

Now I will ask you questions concerning your general health

	In the last two week have you experienced any of the following illness?		
4.1	Fever	[1] YES	[2] NO
4.2	Cough	[1] YES	[2] NO
4.3	Diarrhea	[1] YES	[2] NO
4.4	Vomiting	[1] YES	[2] NO
4.5	Fits	[1] YES	[2] NO

4.6	Stomachache	[1] YES [2] NO
4.7	Skin rash	[1] YES [2] NO
4.8	Difficulty breathing	[1] YES [2] NO
4.9	Loss of appetite	[1] YES [2] NO
4.10	Pain in one breast	[1] YES [2] NO
4.11	Pain in both breasts	[1] YES [2] NO
4.12	Other, specify	_____ _____ _____
4.13	Is there any specific disease that you have been diagnosed of in the last one month in the hospital? **IF NO, GO TO QUESTION 4.15**	[1] YES [2] NO
4.14	If YES above which one	_____ _____
4.15	Are you under any kind of medication (s) currently? **IF NO GO TO QUESTION 4.17**	[1] YES [2] NO
4.16	If YES above which ones **[CONFIRM FROM CLINIC CARDS/PRESCRIPTIONS GIVEN]**	_____ _____ _____ _____ _____ _____
4.17	Are you being provided with any form of food supplements for your own consumption (eg. given in the hospital)? **IF NO, GO TO QUESTION 4.1**	[1] YES [2] NO
4.18	**IF YES,** which type of food are you given?	_____ _____

	In the last one week, in how many days have you experienced the following?	WRITE NUMBER (0-7). IF NOT EXPEREINCED, INSERT ZERO
4.19	Feeling of sadness	[ ]
4.20	Loss of interest in your duties	[ ]
4.21	Loss of appetite	[ ]
4.22	Loss of sleep (sleeping problems)	[ ]
4.23	Loss of thinking/concentration	[ ]
4.24	Feeling of guilt (or worthlessness)	[ ]
4.25	Feeling of excessive fatigue	[ ]
4.26	Difficulty in movement	[ ]
4.27	Suicidal feeling	[ ]

12.0 INFANT MORBIDITY AND MEDICATIONS

Now I will ask you questions concerning the health of [NAME OF INFANT]

	In the last two week have [NAME OF INFANT] experienced any of the following illness?		
5.1	FeveR	[1] YES	[2] NO
5.2	Cough	[1] YES	[2] NO
5.3	Diarrhea	[1] YES	[2] NO
5.4	Vomiting	[1] YES	[2] NO
5.5	Fits	[1] YES	[2] NO
5.6	Stomachache	[1] YES	[2] NO
5.7	Skin rash	[1] YES	[2] NO
5.8	Difficulty breathing	[1] YES	[2] NO

5.9	Loss of appetite	[1] YES [2] NO
5.10	Other, specify	
5.11	Is there any specific disease **[NAME OF INFANT]** has been diagnosed of in the last one month in the hospital? **IF NO, GO TO SECTION 6**	[1] YES [2] NO
5.12	**IF YES** above which one	
5.13	Is **[NAME OF INFANT]** under any kind of medication (s) currently? **IF NO, GO TO SECTION 6**	[1] YES [2] NO
5.14	If YES above which ones **[CONFIRM FORM CLINIC CARDS/PRESCRIPTIONS GIVEN]**	_____ _____ _____ _____ _____ _____

13.0 SEASONALITY

Now I will ask you questions concerning seasons and food in your household

6.1 What is agricultural curent season	(5) Pre-harvest (6) Harvest (7) In between harvest and pre-harvest (8) Other (specify)

14.0 HOUSEHOLD FOOD INSECURITY AND ACCESS SCALE

FOR EACH QUESTION, IF THE RESPONSE IS "2 = NO" THEN PROCEED TO THE NEXT QUESTION. OTHERWISE ASK THE QUESTION IN THE RIGHT COLLUMN

	Occurrence Questions **READ RESPONSES FOR THE INTERVIEWEE.**	**Response**	**If Yes, how often did it occur?** **1 = Rarely** (once or twice in the past four weeks) **2 = Sometimes** (three to ten times in the past four weeks) **3 = Often** (more than ten times in the past four weeks)

7.1	In the past four weeks, did you worry that your household would not have enough food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.2	In the past four weeks, were you or any household member not able to eat the kinds of foods you preferred because of a lack of resources? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.3	In the past four weeks, did you or any household member have to eat a limited variety of foods due to a lack of resources? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.4	In the past four weeks, did you or any household member have to eat some foods that you really did not want to eat because of a lack of resources to obtain other types of food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.5	In the past four weeks, did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.6	In the past four weeks, did you or any household member have to eat fewer meals in a day because there was not enough food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.7	In the past four weeks, was there ever no food to eat of any kind in your household because of lack of resources to get food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.8	In the past four weeks, did you or any household member go to sleep at night hungry because there was not enough food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.9	In the past four weeks, did you or any household member go a whole day and night without eating anything because there was not enough food? **READ RESPONSES**	(1) YES (2) NO	(1) Rarely (2) Sometimes (3) Often
7.10	Did you have enough food to eat over the past 4 weeks?		(1) Never (2)Usually not (more days with not enough food)

		(3) Usually (more days with enough food) (4) Always

8. MOTHER'S DEUTERIUM DOSING AND SALIVA COLLECTION DATA	
DAY 0 (To)	
INTERVIEW/DOSING DATE –DAY 0 (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time predose saliva sample taken (24hrs format)	|__|__|:|__|__|
Time deuterium dose given (24hrs format)	|__|__|:|__|__|
Deuterium dose (g)	|__|__|.|__|__||__|__| GRAMS
DAY 1 (T_1)	
DAY 1 DATE (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 2 (T_2)	
DAY 2 DATE (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY3 (T_3)	
DAY 3 DATE (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 4 (T_4)	

DAY 4 DATE (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 13 (T_{13})	
DAY 13 DATE (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 14 (T_{14})	
DAY 14 DATE (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
ANTHROPOMETRY (for each parameter, two measurements should be taken)	
DAY 0 (To)	
Weight (kg) T_o	|__|__|__|.|__| KG |__||__|__|.|__| KG |__||__|__|.|__| KG
Height/ (cm) T_0	|__|__|__|.|__| CM |__|__|__|.|__| CM |__|__|__|.|__| CM
MUAC (mm) T_o	|__|__| .|__|CM |__|__| .|__|CM |__|__| .|__|CM
DAY 14 (T_{14})	
Weight (kg) T_{14}	|__|__|__|.|__| KG |__||__|__|.|__| KG |__||__|__|.|__| KG
Height/ (cm) T_{14}	|__|__|__|.|__| CM |__|__|__|.|__| CM

	|__|__|__|.|__| CM
MUAC (mm) T_{14}	|__|__| .|__|CM |__|__| .|__|CM |__|__| .|__|CM
9. INFANT'S DEUTERIUM SALIVA COLLECTION DATA	
DAY 0 (T_0)	
DAY 0 (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time baseline saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 1 (T_1)	
DAY 1 DATE (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 2 (T_2)	
DAY 2 (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 3 (T_3)	
DAY 3 (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 4 (T_4)	
DAY 4 (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 13 (T_{13})	
DAY 13 (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
DAY 14 (T_{14})	
DAY 14 (DD/MM/YYYY)	|__|__|/|__|__|/|__|__|__|__|
Time saliva sample taken (24hrs format)	|__|__|:|__|__|
ANTHROPOMETRY (for each parameter, take three measurements)	
DAY 0 (To)	
Weight (kg) T_o	|__|__|. |__|__| |__|KG |__|__|. |__|__|__||KG |__|__|. |__|__|__|KG

Length/ (cm) T_0	|__|__|__|. |__|CM |__|__|__|. |__|CM |__|__|__|. |__| CM
MUAC (mm) T_o	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Head circumference	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Bicept skinfold thickness	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Tricep skinfold thickness	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Subscapular skinfold	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Suprailiac skinfold	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Presence of Bipedal oedema	**[1]** YES **[2]** NO
DAY 14 (T_{14})	
Weight (kg) T_{14}	|__|__|. |__|__| |__|KG |__|__|. |__|__|__||KG |__|__|. |__|__||__|KG
lenghth/ (cm) T_{14}	|__|__|__|. |__|CM |__|__|__|. |__|CM |__|__|__|. |__| CM
MUAC (mm) T_{14}	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Head circumference	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Bicept skinfold thickness	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Tricep skinfold thickness	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Subscapular skinfold	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM
Suprailiac skinfold	|__|__| .|__| CM |__|__| .|__| CM |__|__| .|__| CM

Presence of Bipedal oedema	[1] YES [2] NO
In the last 1 day (24 hours), what food have you given the child **[DO NOT READ RESPONSES]**	[1] BREASTMILK ONLY [2] BREASTMILK AND SOLID FOODS [3] OTHER FOODS OTHER THAN BREASTMILK [4] OTHER SPECIFY _____

Any observations or comments (report any observations or incidences eg. estimated spillages of deuterium um)

_____
_____
_____
_____
_____
_____

10. CHILD BODY COMPOSITION DEUTERIUM DOSING AND SALIVA COLLECTION DATA (ON DAY 14)	
Bottle number	|__|__|__||__|
Time deuterium dose given (24hrs format)	|__|__|:|__|__|
Deutérium dosing (amont in grams)	|__|__|.|__|__||__|__| GRAMS
Time 2 hour saliva sample taken – 2 hours after dosing with deuterium (**24hrs format**) $\mathbf{T_2}$	|__|__|:|__|__|
Time 3 hour saliva sample taken- 3 hours after dosing with deuterium (24hrs format) $\mathbf{T_3}$	|__|__|:|__|__|

Annex 2.3: Focus Group Discussions

SIAYA BREASTMILK INTAKE STUDY (SBIS)

FOCUS GROUP DISCUSSION GUIDE QUESTIONS

Background Information

Date (dd/mm/yyyy): |__|__|/|__|__|/|__|__|__|__|

Moderator ID/Name:
|__|__|__|__|/_____

Note taker ID/Name:
|__|__|__|__|/_____

Number of focus group participants: _____
ID mumbers of the participating mothers

MOTHER ID |__|__|__|__| MOTHER ID |__|__|__|__| MOTHER ID |__|__|__|__|
MOTHER ID |__|__|__|__| MOTHER ID |__|__|__|__| MOTHER ID |__|__|__|__|
MOTHER ID |__|__|__|__| MOTHER ID |__|__|__|__|
MOTHER ID |__|__|__|__| MOTHER ID |__|__|__|__|

Introduction: Each person stating his/name.

Welcome & Informed Consent

Read the following paragraph to the respondent in DHOLUO, and ask if they agree to participate.

Read: We would like to thank everyone for coming to this discussion today, we appreciate your time. My name is _____and I working for the University of Nairobi . This is_____, and [she/he] is a note-taker. I would want each of your to introduce yourselves now. Only introduce yourself by first name.

We would like to have a discussion today about people's thoughts and opinions about infant and young child feeding practices in your community. Even if you do not have direct experience with some of the questions or scenarios we would like to know your opinions. Please remember that we value your thoughts on these topics. The information that you provide will help us improve programs in communities like yours; there are no right or wrong answers.

I will keep everything that you tell me private and confidential, and will not talk to other people about what you have said. I will also keep you and your family's names confidential, and not tell anyone that you have talked to me. Your answers will not lead to any favours to be given. If you have any questions about this, you can call us at our office number. The number is 0722-759449. You may flash our number and we will call you back to respond to any questions or concerns you may have. All answers and discussion will be kept private by the note-taker and me, although we cannot promise that other members of this focus group will do the same. We ask that each of you agree to respect each other's privacy once outside of this focus group setting, by not revealing the names of the other group members or the content of our discussion together.

Your participation in this discussion is voluntary and there is no need to answer any question that you do not want to; however, what you do say will be very important to us.

- We have a note taker and he/she is going to take notes on what we are going to discuss
- We will now explain the structure of this discussion.
    - We will only use first names in the discussion.
    - You do not need to speak in order, but only one person should speak at a time. It is important that everyone be able to hear each other so that you can have a group discussion.
    - We would like to hear from everyone. It is important that you share your ideas with the group. If you agree or disagree with what other people say then please tell that to the group.
    - It is important that there be a true group discussion. Please talk to the whole group not the person seated next to you.
    - I am here to facilitate the group but I am not an expert on the topics. The reason for being here is to hear your thoughts and opinions.
- We think that this group should last between 1 hour and one and half hours. We look forward to hearing your thoughts and opinions whatever they may be.

- I will go around the room and ask your consent to participate. Please say yes or no. Your participation is voluntary and you are free to leave at any time. However, we hope that you will stay for the whole discussion because your thoughts and opinions are valuable to this project.
- **Exclude those who say No. Thank them for coming and let them go.**
- We will begin the discussions now..

6 weeks FGD GUIDE QUESTIONS

1. In the community you live, how do women and men value breastfeeding? **[Prompt: guide the participants to discus about what they see as the importance of breastfeeding]**
2. How in the community is guidance and advice on breastfeeding provided or given? **[Prompt: Also inquire as follow up question, who provides the guidance and advice]**
3. What hinders mothers from initiating breastfeeding within an hour after giving birth? **[Note that: the recommended practice is that mothers should put their newborns to the breast to breastfeed immediately or within an hour after birth]**
4. Which beliefs and cultural practices are related to initiating breastfeeding within an hour after birth? **[If HIV issues comes up, then can record under guide question 5 below, then inquire more about HIV when questions 5 is being asked]**
5. What beliefs are there regarding HIV in the community that hinders mothers from initiating breastfeeding within an hour after giving birth?

Now we want to discus about exclusive breastfeeding **[Explain what exclusive breastfeeding is: Giving the baby no other food or drinks (even water) apart from breast milk for the first 6 months of the babies life. Medicines given are not considered as food]**

6. What does the community know about exclusive breastfeeding for the first 6 months? **[Prompt: guide the participants to discuss about what the community knows about exclusive breastfeeding. If they say it is good or bad, ask the participants to give reasons why it is bad or good]**
7. What reasons would make the mothers not to exclusively breastfeed young children (like those you have) in your community? **[If HIV issues comes up, then record the discussions under guide question 9]**
8. For mothers who are HIV-positive and have young children (like those you have), what hinders them from exclusively breastfeeding?
9. Which reasons are given by mothers and others in the community for not breastfeed young children demand frequently or whenever they demand?

ANNEX 3: STANDARD OPERATION PROCEDURES

Annex 3.1: Standard Operating Procedures for Anthropometric Measurements

Anthropometry Standard OPeration procedures

INFANT ANTHROPOMETRY

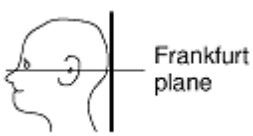
MEASUREMENT OF LEGHTH

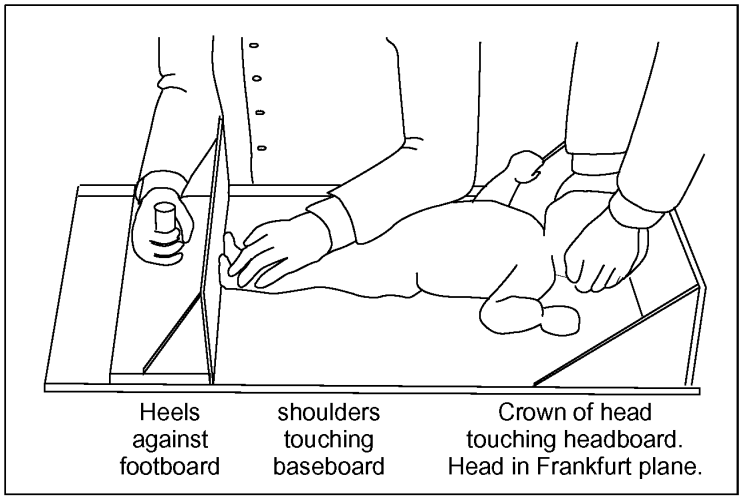
Measuring Recumbent length

Only children less than 24 months will be enrolled in the Winfood study, therefore always the recumbent length will be measured with a measuring board. Two examiners are required to correctly position the subject and to ensure accurate and reliable measurements of length.

1. Place the subject, face upward, with the head at the fixed end of the board and the body parallel to its long axis. The shoulders should rest against the surface of the board.
2. Apply gentle traction to bring the crown of the subject's head into contact with the fixed headboard and simultaneously position the head so that it is in the Frankfurt plane.
3. Hold the subject's feet, without shoes, toes pointing directly upward, while keeping the subject's knees straight by placing one hand on the knees. Then bring the movable footboard to rest against the heels.
4. Record the length to the nearest millimeter.
5. Repeat 1-4 two times, repositioning the subject between measurements.
6. Record all three measurements on the form.

Note: If the subject is restless, only the left leg should be positioned for the measurement.





2. MEASUREMENT OF WEIGHT

Measuring weight with Uniscale

1. Put the scale on the floor. Choose the flattest, most level surface you have.
2. Do not stand on the scale yet.
3. Turn on the scale. Move your foot across switch window **(Figure a)**
4. Ask the mother to step on the scale by herself. She can give her child to you or another person to hold **(Figure b)**
5. Make sure her feet or clothes do not cover the switch window. You will see the mother's weight in the display, for example: 52,4
6. With the mother on the scale pass your foot slowly across the switch window **(Figure c)**. Then wait a couple seconds and you will see: 0,0
7. Ask the mother to step off the scale. You should see: --.-
8. Ask the mother to step back on the scale with her child **(Figure d and e)**. You should see the child's weight: 5.4
9. Ask the mother to step off the scale. You should see: --.-
10. Repeat 4-9 two times more.
11. Record all three measurements on the form.


Figure a

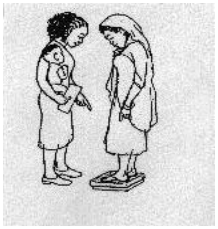


Figure b


Figure c

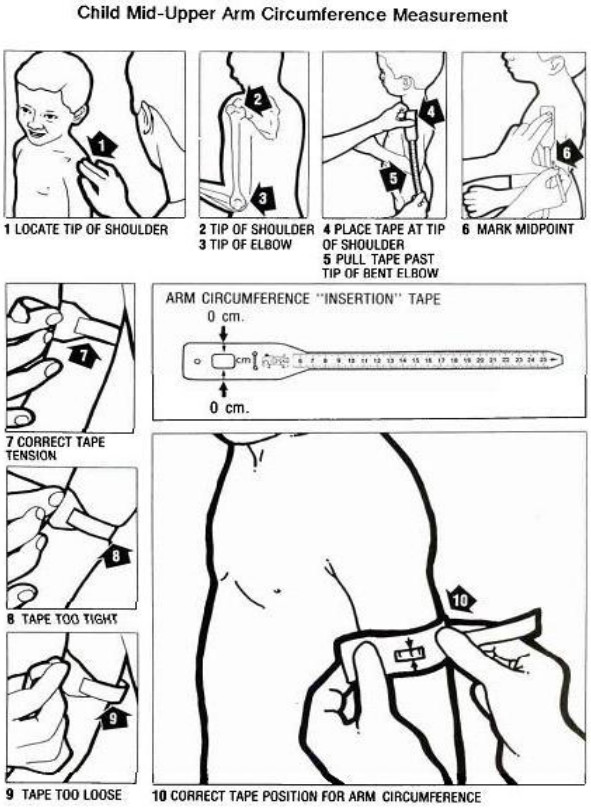

Figure d


Figure e

Measure mid-upper arm circumference

Obtain this measurement with the mother or caregiver seated and holding the infant in her lap. The infant should be wearing loose clothing without sleeves to allow exposure of the shoulder area. Use a fiberglass insertion tape.

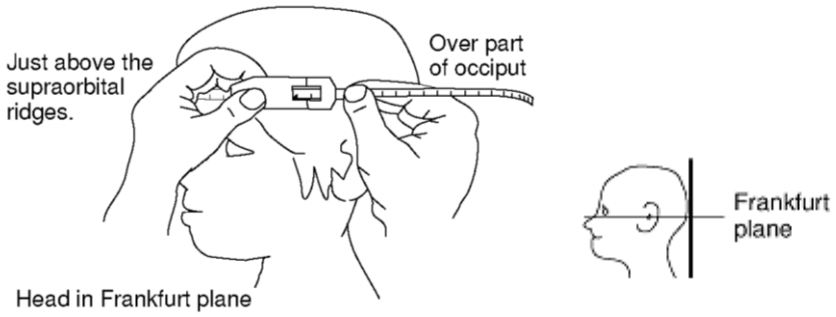
1. Gently bend the left arm through 90 degrees at the elbow, and then place the forearm with the palm down across the body.
2. Locate and mark the tip of the shoulder.
3. Locate the tip of the elbow.
4. Measure the distance between these two points using a fiberglass insertion tape, and mark the midpoint with a soft pen or indelible pencil, directly in line with the point of the elbow and shoulder.
5. Relax the arm so that the elbow is extended and hanging just away from the side of the trunk, with the palm facing the thigh. Then wrap the tape gently but firmly around the arm at the midpoint, care being taken to ensure that the arm is not squeezed. Measurements are taken to the nearest mm.
6. Repeat 1-5 two times more.
7. Record all three measurements on the form.



Measure head circumference

For the measurement, a fiberglass insertion tape. Any added objects in the hair such a hair pins should be removed for the measurement.

1. The measurer should stand facing the left side of the subject. Gently, place the subject, face upward, on an examination table, allowing the shoulders to rest against the surface of the table.
2. Position the head so that it is in the Frankfurt plane i.e., an imaginary plane which passes through the external auditory meatus (the small flap of skin on the forward edge of the ear) and over the top of the lower bone of the eye socket immediately under the eye, is vertical.
3. Place the tape just above the supraorbital ridges covering the most prominent part of the frontal bulge and over the part of the occiput that gives the maximum circumference. Care must be taken to ensure that the tape is at the same level on each side of the head and pulled tightly to compress the hair.
4. Measure the circumference to the nearest millimeter.
5. Repeat 1-4 two times more.
6. Record all three measurements on the form.



Measure skinfold

Obtain these measurements with the mother seated and holding the infant in her lap.

Alternatively, children may be measured lying down. It is helpful to demonstrate the caliper on the hand of the measurer and on the hand of the infant, measuring total palm thickness, before beginning to measure skinfold thickness.

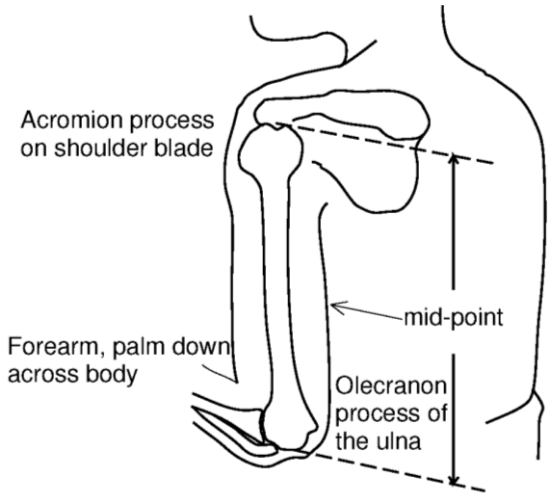
Measure the tricepskinfold

Gently bend the left arm through 90 degrees at the elbow, and then place the forearm with the palm down across the body.

Locate and mark the tip of the acromion process of the shoulder blade at the outermost edge of the shoulder.

Locate the tip of the olecranon process of the ulna.

Measure the distance between these two points using a fiberglass insertion tape, and mark the midpoint with a soft pen or indelible pencil, directly in line with the point of the elbow and shoulder.



Extend the infant's arm so that it is hanging loosely by the side.

Grasp a vertical fold of skin plus the underlying fat, 1cm above the marked midpoint, in line with the tip of the olecranon process, using the thumb and forefinger.

Gently pull away the skinfold from the underlying muscle tissue, and apply the caliper jaws at right angles, exactly at the marked midpoint.

Hold the skinfold between the fingers while measuring.

Repeat 5-8 twice more and write the measure in the form

Measure subscapularskinfold

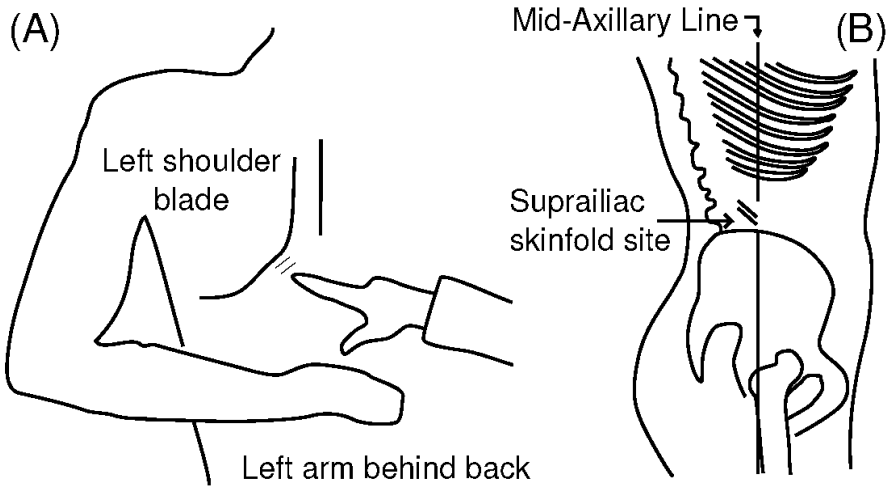
The site is just inferior to the inferior angle of the shoulder, and can be identified more readily by placing the child's arm behind the back (see figure A).

To locate the site, the health professional will run a finger along the shoulder blade until the inferior angle is identified.

Relax the shoulder and the arm, and pick up a skinfold on a 45^0 angle from horizontal, in the same direction as the inner border of the shoulder (i.e., medially upward and laterally downward) (See Figure A).

Skinfolds should be recorded to 0.2 mm on the Harpendenskinfold calipers three times. Skinfold measurements made with precision calipers should normally agree to within 1mm.

Record all 3 measurements on the form.



MEASUREMENT OF THE BIPEDAL OEDEMA

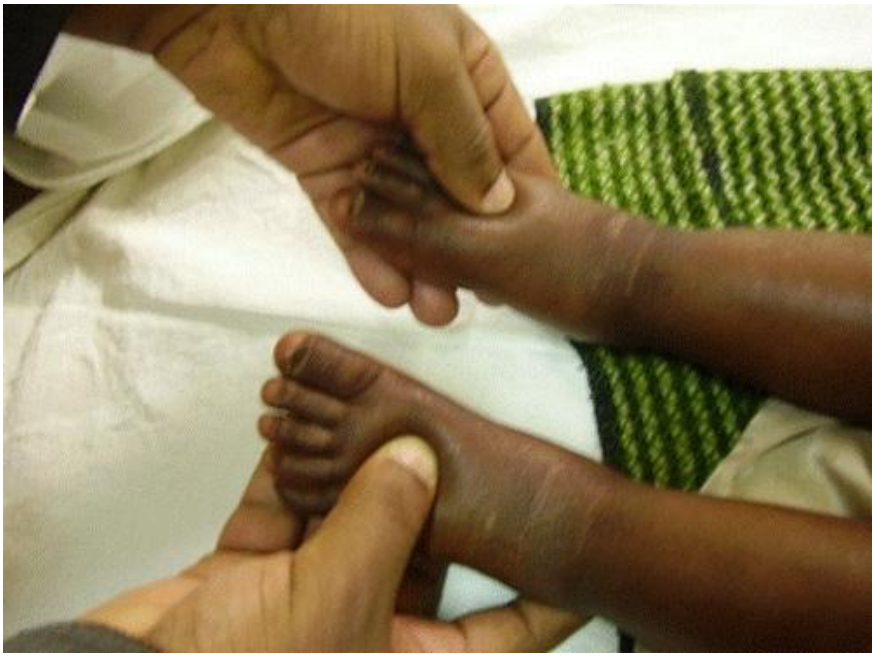
Introduction

Oedema is the presence of excessive amounts of fluid in the intracellular tissue and a sign that the body is lacking protein balance arising from dietary inadequacy. When this occurs in both feet the condition is termed as bi-pedal oedema and an indicator of severe malnutrition that needs urgent attention to correct the deficiency

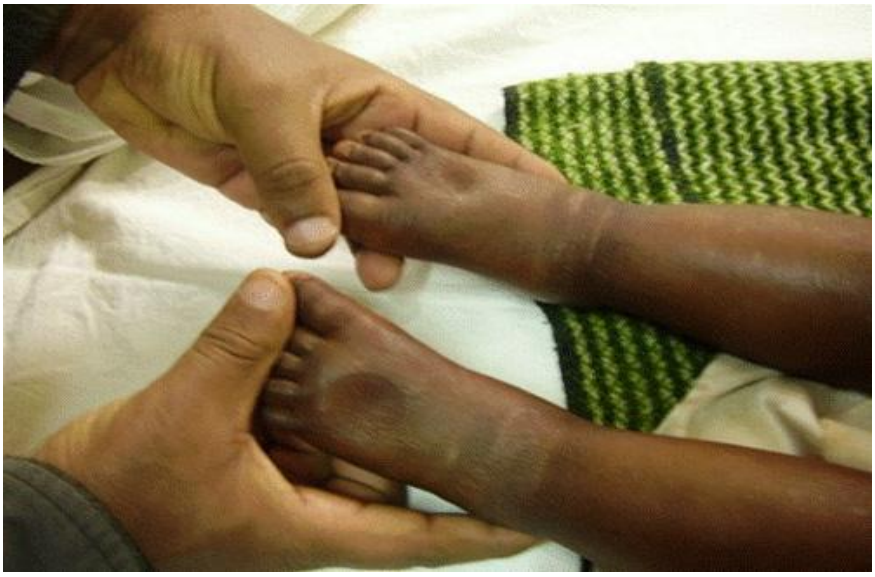
How to identify bi-pedal oedema (see figure below)

1. Apply moderate thumb pressure for about three seconds to the tops of the feet or ankle on both legs. If you count “one thousand and one, one thousand and two, one thousand and three” in English, pronouncing the words carefully, this takes about three seconds.

2. The impression of the thumb will remain for some time when oedema is present; oedema is diagnosed ONLY if both feet show the impression for some time. This is a clinical sign of severe malnutrition,



Applying moderate pressure to both feet.



Impression of thumb persists for some time

Take note of the following;

When a child has oedema, it is automatically included with children counted as severely malnourished, independently of its wasting status.

This is due to the strong association between oedema and mortality.

5.4 How and when to refer children for specialised care

Use the standard referral card (Annex A) whenever you are making referral; refer all children who meet any of one or both of the above criteria.

Always refer to the nearest health centre.

A. ANTHROPOMETRY FOR THE MOTHER

1. HEIGHT OF THE MOTHER

1. Measurer or assistant: Place the measuring board on a hard flat surface against a wall, table, tree, staircase, etc. Make sure the board is not moving.

2. Measurer or assistant: Ask the mother to remove shoes and unbraid any hair that would interfere with the height measurement. Ask her to walk to the board

3. Assistant: Place the questionnaire and pencil on the ground (Arrow 1). Kneel with both knees on the right side of the mother (Arrow 2).

4. Measurer: Kneel on your right knee on the mother's left side (Arrow 3). This will give you maximum mobility.

5. Assistant: Place the mother's feet flat and together in the centre of and against the back and base of the board/wall. Place your right hand just above the mother's ankles on the shins (Arrow 4), your left hand on the mother's knees (Arrow 5) and gently push against the board/wall.
Make sure the mother's legs are straight and the heels and calves are against the board/wall (Arrows 6 and 7). Tell the measurer when you have completed positioning the feet and legs.

6. Measurer: Tell the mother to look straight ahead at the mother who should stand in front of the mother. Make sure the mother's line of sight is level with the ground (Arrow 8). Place your open left hand under the mother's chin.
Gradually close your hand (Arrow 9). Do not cover the mother's mouth or ears. Make sure the shoulders are level (Arrow 10), the hands are at the mother's side (Arrow 11), and the head, shoulder blades and buttocks are against the board/wall (Arrows 12, 13, and 14). With your right hand, lower the headpiece on top of the mother's head. Make sure you push through the mother's hair (Arrow 15).

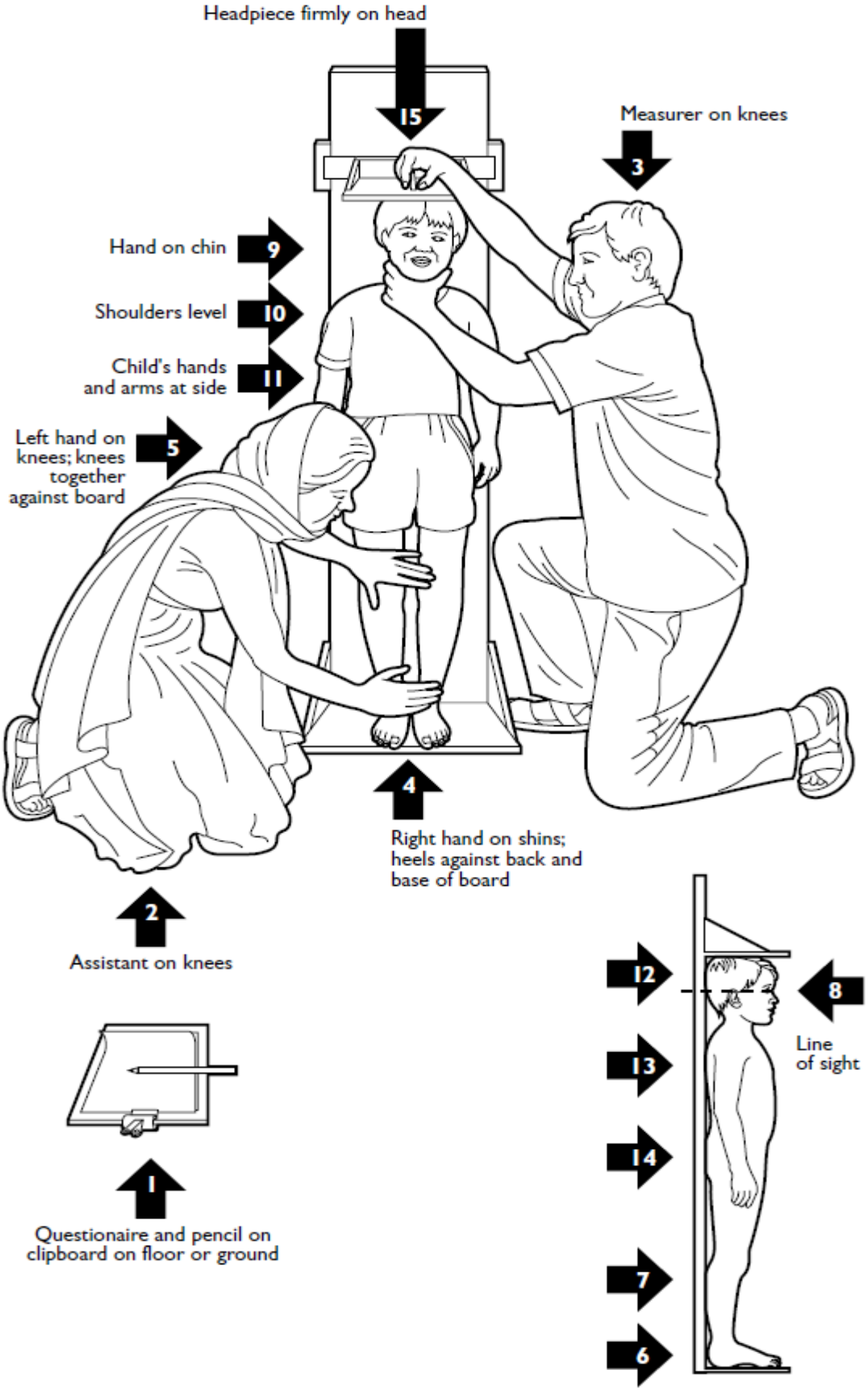
7. Measurer and assistant: Check the mother's position (Arrows 1-15). Repeat any steps as necessary.

8. Measurer: When the mother's position is correct, read and call out the measurement to the nearest 0.1 cm. Remove the headpiece from the mother's head and your left hand from the mother's chin.

9. Assistant: Immediately record the measurement and show it to the measurer.

10. Measurer: Check the recorded measurement on the questionnaire for accuracy and legibility. Instruct the assistant to erase and correct any errors.

11. Measurer; Repeat the measurement two times. If the readings differ by more than 0.5 cm, measure the mother a 3rd time


Headpiece firmly on head
15
Measurer on knees
3
Hand on chin
9
Shoulders level
10
Child's hands and arms at side
11
Left hand on knees; knees together against board
5
4
Right hand on shins; heels against back and base of board
2
Assistant on knees
1
Questionaire and pencil on clipboard on floor or ground
12
8
Line of sight
13
14
7
6

Taking height of mother (an adult)

2. WEIGHT OF THE MOTHER

1. Prepare mother for weighing:
   - Mothers should be asked to remove any heavy outer clothing, sweater, etc.
   - Mothers should also be asked to remove shoes before they step on the scale.

2. Prepare the scale:
   - Ensure that the scale is still on level ground.
   - Check that the scale is reading "zero" before the mother steps on the scale.

3. Weigh the mother:
   - Ask the mother to step on the scale and hold still.
   - Records weight on the form.
   - Ask the mother to step off the scale.

4. Repeat the measurement process one more times; be sure the scale reads "zero" each time before the mother steps back on the scale.

3. MUAC OF THE MOTHER

As for the measurement of the MUAC of children.

Annex 3.2: Standard Operating Procedures for Breast Milk Intake Measurements

SALIVA SAMPLES COLLECTION FOR BREASTMILK INTAKE MEASUREMENTS

STANDARD OPERATION PROCEDURES

Step I: Saliva (T_0)

Using a ball of sterile cotton wool and syringe, collect pre-dose saliva from mother and baby pair (this procedure is important to avoid agitating baby too early). Mother should have fasted overnight before baseline saliva (only if body composition measurement is of interest).

Saliva samples should not be collected if she has eaten or drank anything in the last 30minutes.The vial label should consist of date of collection, time, and mother's code number. All saliva vials should bear sample e.g. T_0, T_1, T_2 etc

Dosing procedure

Using a standard straw the mother drinks the pre-weighed deuterium dose from bottle

Rinse the bottle with about 10-15 ml drinking water and ask mother to drink.

Step II: Anthropometric data

Take mother's anthropometric data as the second step. Mother should be asked to remove shoes and excess clothing before measurements are taken. Using an electronic scale, record mother's weight to 0.1kg. Take her height using a standard height board.

Mid-upper-arm circumference (MUAC): Using a MUAC tape, take the length of upper arm (with elbow bent and arm relaxed) and divide by 2 to get middle of the upper arm. Then take length up to the actual middle. Tie the tape round the mid-upper and precisely record circumference to nearest cm.

Baby's anthropometric data: Use the MUAC tape to take baby MUAC and head circumference to nearest cm. Place the baby on the length board and record length (height) to nearest cm. Enumerator should ask the mother to help undress baby for weight measurements. Using an electronic scale weigh the baby to nearest 0.01kg (initial wt). The scale basin should be lined with a clean cotton cloth to avoid chilling of baby.

Step III: Subsequent saliva sampling – Days 1,2,3,4, 13 and 14
Explain clearly to mother the schedule of visits for saliva sampling. The mother should provide a reliable telephone contact for follow up reminders (or landmark features to her home where she can be reached)
Timing of saliva sampling schedule should be consistent i.e. T_1 T_2 etc, should be collected at approximately the same time of the day as T_0 was collected

Subject and sample handling

- Use a clean pair of surgical gloves for each subject
- Sample vial must bear all information –
  1.Mother/baby pair code No.
  2.Date
  3.Time code (e.g. T_0)
  4. Time of sampling (in 24hr format)
- Store each sample in a small plastic zip-lock bag
- Pool all corresponding samples according to time code (e.g. T_{0s} for mothers together) and subsequent for the rest. Put the bags into a paper bag and store in cool box with ice packs
- Transport the day's samples to a freezing facility (-20^{o}C) where they should stay until exercise is completed.
- Transport all the samples in frozen form to the FTIR laboratory for analysis

NB:
**Baby weight must be taken again on day 14 as final weight*

Labeling of the vials

a. **Mother code**: 001M
b. **T-Number** T0
c. **Date in British system**: 15021014
d. **Time in 24 hours:** 13:40

Annex 3.3: Standard Operating Procedures for Body Composition Measurements

SALIVA SAMPLES COLLECTION FOR BODY COMPOSITION MEASUREMENTS

STANDARD OPERATION PROCEDURES

Background and objective of body composition measurement: The aim of the study is to equip enumerators with skills on performing stable isotope-based technique for field assessment of body composition among breastfed infants.

Importance of body composition: Explain to both enumerators that amount of fat in a child's body is important for child growth, development and health, that some kind of fat may be associated with some diseases in some individuals as they grow older. The type of food eaten by children determines the type of fat in the body. Measuring body composition (amount of fat in the body) enables determination of adequacy of foods on providing essential fat for growth and also prediction of later health status.

Safety of stable isotope: Deuterium oxide already exists in the human body in small quantities and is thus not toxic at all. It a stable form of ordinary water.

Maternal/caretaker consent: Obtain written consent from the mother/caretaker before proceeding with next steps. If mother/caretaker declines invitation, explain that it is voluntary and their refusal will not affect how they are treated.

Baseline demographic information: The interviewer will then ask mother to provide her socio-demographic information (date of birth, marital status and income) and also the baby's date of birth and sex. This information should be collected using a standard data sheet. Interviewee will then alert the mother of the next step and explain how it is done.

What does the technique entail?: The process is done by giving a one-time dose of deuterium oxide solution to the infant and analyzing its disappearance from the child's fluids (saliva or urine). The amount of deuterium dose given to each child depends on their weight, which must be measured first. This is followed by sampling of pre-dose saliva from the child. Once weight and pre-dose saliva samples are determined, an amount of deuterium solution is weighed out at a rate of 3 grams for babies less than 10 kgs. Babies weighing between 10kg and 20 kgs will be dosed with 6 grams of deuterium. The child is given the dose from a syringe or straw as appropriate while avoiding any spillages. After dosing the child with deuterium their saliva are sampled over a period of 3 hours at 0hr and 2hr and 3hr, respectively. The samples are clearly labeled to reflect date and time of sampling thus: T_0 (Pre-dose), T_2 (2 hours) and, T_3 (3 hours).

Child preparation and weight measurement

Ask the mother the last time the child was fed/breastfed to ensure that the child has not been fed in the last 30 minutes prior to saliva sample collection; if they have been fed proceed with weight measurement, but hold on with pre-dose sampling and dosing until at least 35 minutes.

Weigh the child with minimal clothing using calibrated weighing scale, taking two measurements, and record to the nearest 100g (1 decimal point e.g. 8.0 kg NOT 8 kg)

Dosing and sample collection schedule

On this day baseline information, anthropometry are taken and child given deuterium oxide dose to drink.

The researcher;

1. Explains to the participant what to expect throughout the study
2. Obtain informed written consent from mother/caretaker
3. Collects the initial demographic details of the participant
4. Takes the weight, length and head-circumference measurements for the baby
5. Collects baseline saliva for infant (try as much to work with the mother to allow her to assist you with the baby as this helps to enhance calmness) – mark this as $T_{0,}$ center code and subject code, date and time. Place vial in a rack.
6. Dose the infant with the entire pre- weighed deuterium water, using a syringe. Do not discard the dose bottle. Record dose amount on participant's data sheet.
7. Rinse the bottle with about 15mls of clean drinking water. Let the infant drink that water to ensure all the deuterium is washed from the bottle. Repeat this step.
8. Allow two hour equilibrium period and collect sample saliva $T_{2.}$

T_2: Take samples of saliva from infant after 2 hours

T_3: Collects saliva from infant after 3 hours

How saliva sample is obtained

The researcher:

1. Must wear hand gloves (Note: wear different hand glove for each mother-infant pair).
2. Prepares sterile cotton wool ball
3. Inserts the cotton wool ball into infants mouth aiming to position it under the tongue
4. The cotton ball is rolled gently around the mouth by the researcher/mother until the cotton is soaked with saliva
5. Removes the wet cotton ball from infant's mouth, puts it into a clean syringe barrel
6. Squeeze out the saliva into a vial and label

How sample tubes are labeled.

2. Note the center code and participant as follows
    a. **Mother code**: 001M
    b. **T-Number** T0
    c. **Date in British system**: 15021014
    d. **Time in 24 hours:** 13:40
3. Indicate the HOUR of sampling as T_0, T_2,T_3 (For example center code-participant code-T_0)
4. Indicate the sampling time in **24 hour format**, for example 13.45 hr
5. Keep the samples in zip-locked bags which must also be labeled (Note: keep T_0, T_2, T_3 samples in separate zip locks to avoid contamination since deuterium enrichment at a particular hour is different)

Sample storage and handling

1. Samples in zip lock bags are kept in cooler box with ice
2. Aim to deliver samples from field to the station within 2-3 hours
3. At the station store samples in a freezer at -20 ^{o}C
4. Transport samples to analyzing laboratory in frozen state inside ice-cooled boxes

Equipment check list

The following materials must be available to make this work possible

1. Data recording sheet/questionnaire
2. Pen and extra notebook
3. Weighed deuterium oxide in well sealed bottles
4. Indelible marker pen
5. Label stickers
6. Syringes (10 and 20 mls)
7. Sample vials tubes (4 ml)
8. Hand gloves (sterile, without powder)
9. Clean drinking water (prefer mineral water)
10. Infant weighing scale (e.g. Salter scale)
11. Length board/mat for infants
12. Non-stretchable measuring tape, MUAC tapes
13. Zip-lock bags
14. Plastic bags
15. Cotton wool
16. Cooler box with ice packs
17. Rubber bands

Annex 3.4: Standard Operating Procedures for FTIR Measurements

FTIR analysis of saliva samples for Deuterium Oxide (D2O) CONCENTRATION

- Remove saliva samples from freezer and place them on bench (on a rack) to allow thawing
- Ensure that the samples are sorted (IN A SEQUENCE) according to subject's code e.g. for body composition measurements, each subject (code or ID) has 3 saliva samples labeled T0, T2 and T3. They should be analyzed in that sequence. For Breast milk intake measurements, each subject (code or ID) has seven samples (14 samples for mother/baby pair) labeled T0, T1, T2, T3, T4, T13 and T14. They should be analyzed in that sequence
- Start the FTIR machine (about 40 minutes) before switching the computer on. This gives machine time to warm up. (Note: If you switch on computer before machine, the computer software will not recognize the FTIR software). FTIR is ready for use when the 2 green lights (interface and mirror) appear on the top right hand side of the screen
- Use a micro syringe (1ml) to load sample into clean cell and place it carefully into FTIR chamber. Ensure the sample holder (cell) is clean before loading the sample. Use clean (bottled) water to cleanse the cell each time before loading a new sample
- Always start with background (BKG), which is basically clean bottled water from supermarket. This is the BKG for standard Deuterium (D2O)
- Click on 'Measure' then BGK. Allow time for scanning (32 scans) until peak has stabilized
- Remove sample holder and suck out the sample using a clean micro syringe
- Load Deuterium standard and measure as 'sample'
- Go to file and select "EXPORT". This process will export the FTIR reading to the MRC ISOTOPE software
- Remove sample holder cell from chamber and micro syringe to remove standard from sample holder, and rinse the cell using clean water from bottle. Rinse several times to avoid "contamination" of the next sample with standard D2O
- Repeat procedure for samples, i.e. T0, T2 and T3 for each individual subject (body composition measurements) or T0, T1, T2, T3, T4, T13 and T14 (breast milk measurements). T0 is the BKG for that sequence of samples
- Go to the MRC ISOTOPE software and load sample absorbance against the Standard D2O loaded earlier. This step will give the concentration of D2O in the saliva sample as parts per million (**ppm)**
- Record concentration of D2O in saliva sample as it appears on the screen

Important Practice:

- Use mechanical mixer to aspirate the samples to homogenous mix. This step is important to eliminate fractionation of deuterium oxide in the sample
- Wear surgical gloves before handling samples
- Use a new pair of surgical gloves for each sample; to avoid "contamination" of samples with D2O of previous sample
- Always rinse sample holder cell several times with clean bottled water (before loading sample) to avoid "contamination" of the next sample with D2O from previous sample
- Use clean tissue (or Kim wipes) to wipe cell and surfaces and maintain cleanliness throughout the analysis
- Always ensure that Measurement is complete at 32 scans
- Ensure to select a unique file name for each sample

Annex 3.5: Standard Operating Procedures for Holding Focus Group Discussions
SIAYA BREASTMILK INTAKE STUDY (SBIS)

SOP IN CONDUCTING THE FOCUS GROUP DISCUSSIONS WITH MOTHERS

A. Introduction to FGD

A focus group is a qualitative data collection method in which one or two researchers and several participants meet as a group to discuss a given research topic. These sessions are usually tape recorded, and sometimes videotaped. One researcher (the moderator) leads the discussion by asking participants to respond to open-ended questions – that is, questions that require an in-depth response rather than a single phrase or simple "yes" or "no" answer. A second researcher (the note-taker) takes detailed notes on the discussion. A principal advantage of focus groups is that they yield a large amount of information over a relatively short period of time. They are also effective for accessing a broad range of views on a specific topic, as opposed to achieving group consensus.

B. Selection of the 10 participants per FGD participants

The selection of those mothers who have completed day 14 of the deuterium should be random. This should be done by placing papers (with the mother ID) in containers, shaking and drawing one at a time. Re-shaking should be done after each draw. Two groups to be considered

i. Reactive group
ii. None-reactive group

C. Mobilising the 10 participants

Calling 3 days before the interviews and inform about:

i. The purpose of the coming – to have a discussion about child feeding and care
ii. The date and time of the discussion
iii. Approximate time that will be taken- 45min- 1 hour
iv. The refund of the fare used
v. The time of the meeting 11-12.30am. Then they are released to go home.
vi. Ask them if they will agree to give views about young child feeding in their communities.

D. Consenting of the participant

i. Consent forms and the FGD guide questions need to be translated to dholuo.

ii. Consenting need to be done in the group – Read the consent form when they are together

E. Facilitating of the FGD

Asking open-ended questions

Closed-ended questions are questions that may be answered with a single word or phrase, or with a "yes" or "no" response. An example is, "Have you ever used the family planning services at xxxx clinic?" It is difficult to glean much insight from these brief responses, because they usually do not indicate "why" or "how." A better technique for getting in-depth answers is to phrase questions as open-ended – that is, requiring more than a "yes" or "no" response. Open-ended questions set no limits on the range or length of responses, instead giving participants the opportunity to explain their position, feelings, or experiences. An example is, "What were your experiences using the family planning services at xxxx clinic?"

What are leading questions and how do I avoid them?

Leading questions are questions worded in such as way as to influence participants' responses – in other words, questions that lead participants along a particular line of thinking. Asking leading questions risks conveying your own value judgments and biases and imposing a perspective on participants. When asked a leading question, participants are likely to provide a response those accords with it simply because they are reluctant to contradict the moderator. To avoid this, ask neutral questions free of preconceptions.

What are follow-up questions and how do I use them?

Follow-up questions (or sub-questions) are intended to ensure that participants provide the complete set of information each main question was designed to elicit. They prompt participants to speak on some aspect that was not mentioned in response to the original question. Sub-questions are often provided in the focus group guide under each main question or topic, as cues for the moderator. If a participant answers a sub question in the initial response, it is not necessary to then pose that sub-question. Engaged listening will help you decide which follow-up questions to ask.

What are probes and how do I use them?

Probes are neutral questions, phrases, sounds, and even gestures moderators can use in focus groups to encourage participants to elaborate upon their answers and explain why or how. Suggestions for probes are sometimes outlined in the focus group guide, but they are also left to the discretion of the moderator. The particular probe used depends on the response given by the

individual participant. Probing therefore requires the moderator to listen carefully to participants and to engage actively with what they say. You should use probes when a participant's response or contribution is brief or unclear, when a participant or the group seems to be waiting for a reaction from you before continuing to speak, or when a person appears to have more information on the subject. As much as possible, probe for more detail about what the participant thinks, feels, and experiences in relationship to the research topic. Do not assume that you understand the intent of a brief response. Instead, use probes to further or confirm your understanding and to encourage more explanation. Be careful, however, not to use probes to excess. Balance knowing when to probe with knowing when to move on to the next question. If responses are repetitive or lacking in substance, or if the participant becomes annoyed or upset about lingering on a particular topic, it is best to advance to the next question. Probing is probably the most important technique in focus group moderation, but also the hardest to master. It requires practice, thorough knowledge of the focus group guide and research objectives, and a solid understanding of what kind of information each question is intended to elicit. It also requires patience and sensitivity, effective time management, and good interpersonal skills.

F. Note taking of the FGDs

Create a form on which to write your notes. If a note-taking form is not provided, creating one can help you organize your notes during the session and make it easier to expand your notes. For example, you might have several columns – one to identify the speaker, another to write quotes or the main idea of what a speaker said, and another in which to write your observations. Begin each notebook entry with the date, time, place, and type of data collection event, and either leave space on the page for expanding your notes, or plan to expand them on a separate page.

Take notes strategically. It is usually practical to make only brief notes during data collection. Direct quotes can be especially hard to write down accurately. Rather than try to document every detail or quote, write down key words and phrases that will trigger your memory when you expand notes. However, remember that your notes will be the only documentation of the session if the recording fails or is faulty. Try to capture the content of all essential verbal contributions, and when possible, to document especially representative quotes word-for-word.

Record participant identifiers. It can be a great help during later transcription if you note the identifier of each participant as they speak. The moderator can make this easier for you by asking participants to say their identifier before making a contribution.

Use shorthand. Because you will expand and type your notes soon after you write them, it does not matter if you are the only person who can understand your shorthand system. Use abbreviations and acronyms to quickly note what is happening and being said.
Record both the question and the response. If the question or probe comes from a focus group question guide, save time by noting the question number. If it is not possible to record direct quotations, write down key words and phrases.

Distinguish clearly between participant comments and your own observations. You could use your own initials or "MO"to indicate "my observation." For example:"MO – embarrassed by empty beer bottles in room." This documents the researcher's observation that the participant seemed embarrassed about the empty beer bottles in the room.

Cover a range of observations. In addition to documenting what people say, note as well as you can their body language, moods, or attitudes; the general environment; and other information that could be relevant.

Debriefing sessions
The note-taker typically conducts a debriefing session with the moderator immediately after the focus group. This should ideally begin shortly after the discussion session has ended, say within 15 minutes or a half-hour. Although the mood should be more relaxed than during the discussion session, it should not be completely informal. Debriefing is a very important part of focus group research and must be done with a certain degree of rigor to maximize its usefulness.

Debriefing has multiple purposes:

a. To log any additional information about the focus group while it is still fresh in the memory. For example, even when audiotapes are used to record the session, there will be nonverbal communication, such as gestures, facial expressions, eye contact, tension, that will not be picked up on tape.

b. To discuss issues or comments that needs clarification. Field notes explaining confusing parts of the focus group will be valuable for helping other researchers to interpret the transcripts later on.
c. To discuss particular questions that did not work well and why.
d. To note any information that contradicts or confirms data collected in previous sessions.
e. To discuss new topics that arose during the focus group.

f. To identify missing information. Comparing what information was being sought with what was actually learned can help moderators plan how to solicit this information more effectively in subsequent focus groups.
g. To identify information that needs to be researched outside the focus group setting. This may have to do, for example, with cultural norms, fact-checking, or specifics about the study.
h. To discuss trouble spots that came up during the focus group, with regard to participants, group dynamics, and questions. It may be necessary to develop new strategies for dealing with a particular issue for subsequent focus groups.

To provide the moderator and note-taker a forum for giving each other constructive feedback

G. Filling of the FGD notes

a. The notes of the FGD should be filed accordingly. The filed notes should indicate
    i. The date of the FGDs.
    ii. Names of participants
    iii. The group- Reactive or non-reactive groups

ANNEX 4: UNIVERSITY REGISTERATION

University of Nairobi
Board of Postgraduate Studies

Telephone: 318262
Fax Number: 243626
Telegrams: "Varsity of Nairobi"
Email: bps@uonbi.ac.ke

P. O. Box 30197, 00100
Nairobi, Kenya

Our Ref: 31350/2012

19th December 2012

Oiye Shadrack Okoth
P.O. Box 17094 -00510
NAIROBI

Dear Mr. Oiye,

RE: ELIGIBILITY FOR Ph.D. ADMISSION (PROVISIONAL)

I am writing to inform you that the Director, acting on behalf of the Board of Postgraduate Studies has approved that you are eligible for provisional admission into the Ph.D programme in HIV and Nutrition in the Institute of Tropical & Infectious Diseases for six months with effect from 14th December 2012 to 13th June 2013 to enable you perfect your research proposal entitled: **"Effect of maternal HIV status on breast milk intake and growth of HIV-uninfected Kenyan infants at 6 weeks post-partum and 6 months of age".** She has also approved **Prof. Benson B.A. Estambale, Dr. Victor Owino** and **Prof. Suzanne Filteau** as the supervisors of your thesis.

You are expected to complete the preparation of your PhD proposal, have it approved by the Board of Postgraduate Studies and be entered for full admission before the expiry date given above, failure to which you will cease to enjoy provisional registration. In extenuating circumstances, an extension of provisional admission of up to 6 months may be granted once only.

Please note that upon provision of full PhD registration you will be required to pay full fees as indicated below:

A. COMPOSITE FEES	**Year 1**	**Year 2**	**Year 3**
Registration Fee	**2,000/= p.a.**	**2,000/= p.a.**	**2,000/= p.a.**
ID/Card	**500/= p.a.**	**500/= p.a.**	**500/= p.a.**
Tuition Fees:	**108,700/=**	**108,700/=**	**108,700/=**
Supervision	**12,000/=**	**12,000/=**	**12,000/=**
Exam: (Written)	**12,000/=**	-	-
Computer Fee	**5,000/= p.a.**	**5,000/= p.a.**	**5,000/= p.a.**
Activity Fee	**2,000/= p.a**	**2,000/= p.a**	**2,000/= p.a**
Medical Fee	**5,000/= p.a**	**5,000/= p.a**	**5,000/= p.a**
Caution Money (Once)	**5,000/=**	-	-
	152,200/=	**135,200/=**	**135,200/=**

B. OTHER CHARGES:

1. Extension of registration period - Kshs. 3,000/= p.a.
2. Extension of correction period - Kshs. 1,000= per three months
3. Extension of revision period - Kshs. 3,000/= p.a
4. Extension of supervision - Kshs. 12,000/=
5. Examination of revised thesis - Kshs. 9,000/=

The degree for which you are registered will be offered by research and thesis and in this connection the guidelines for research money are:

Arts Based Research	-	**Kshs. 150,000/=**
Science Research	-	**Kshs. 200,000/=**
Clinicals Research	-	**Kshs. 250,000/=**
Book Allowance	-	**Kshs. 40,000/=**

Please note that all fees and other charges due should be paid by bankers cheque addressed to University of Nairobi Enterprise and Services Limited (UNES). Direct deposits should be made to Account No.03-073-1021554, at any Barclays Bank of Kenya branch.

The bankers' cheques and deposit slips should be receipted at the UNES Finance office, Gandhi Wing in room G3 before reporting to the Board of Postgraduate Studies with a copy of the receipt and three coloured recent passport size photographs of yourself for registration.

As the University does not provide accommodation for postgraduate students, you must make your own accommodation arrangements. We estimate that you will need over Ksh[illegible],000.00 per month to cover this and other personal expenses.

You are advised that all fees and other charges may be subject to change without prior notice.

Yours sincerely,

ANNE M. SIMIYU (MISS)
FOR: DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Cc: Director, UNITID
Prof. Benson Estambale (Supervisor), UNITID
Dr. Victor Owino (Supervisor), UNITID
Prof. Suzanne Filteau (Supervisor), London School of Hygiene & Tropical Med.

AMS/bwg

ANNEX 6: RESEARCH APPROVALS

Annex 6.1: Ethical Approvals, Amendments and

UNIVERSITY OF NAIROBI
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P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link:www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/90

17th April 2013

Oiye Shadrack Okoth
UNITID
College of Health Sciences
University of Nairobi

Dear Mr. Oiye

Research proposal: Effect of maternal HIV status on Breast milk intake and growth of HIV-uninfected Kenyan infants at 6 weeks post-partum and 6 months of age (P517/09/2012)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above revised proposal. The approval periods are 17th April 2013 to 16th April 2014.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN**

Protect to Discover

Yours sincerely

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. Prof. A.N. Guantai, Chairperson, KNH/UoN-ERC
The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
The Director, UNITID, UoN
The HOD, Records, KNH
Supervisors: Prof. Benson Estambale, Dr.Victor Owino, Prof. Suzanne Filteau

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KENYATTA NATIONAL HOSPITAL
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Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/ MOD/75

13th February 2014

Oiye Shadrack Okoth
UNITID
College of Health Sciences
University of Nairobi

Dear Shadrack

Re: Approval of reimbursement study titled - Effect of maternal HIV status on breast milk intake and growth of HIV-uninfected Kenyan Infants at 6 week postpartum and 6 months of age (P517/09/2011)

Your communication dated January 15, 2014 refers.

The KNH/UoN-ERC has reviewed and approved the following modifications:

1. Addition of objective 4 to take care of the body compositions measurements
2. Literature review – brief documentation of the rationale for body composition assessment using deuterium method and available information on the same.
3. Consent form: Additional explanation for the dosing of child for body composition measurement on day 14 of the mother-child visit.
4. Additional tool for capturing body composition measurement.
5. Addition of standard operation procedures

The revised document is hereby endorsed and stamped for use.

Yours sincerely

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. Prof. A.N. Guantai, Chairperson, KNH/UoN-ERC
The Deputy Director CS
The Principal, College of Health Sciences, UoN

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Ref. No.KNH/ERC/R/23

7th March 2014

Oiye Shadrack Okoth
UNITID
College of Health Sciences
University of Nairobi

Dear Shadrack

Re: Approval of annual renewal - study titled – Effect of maternal HIV status on breast milk intake and growth of HIV-uninfected Kenyan infants at 6 week postpartum and 6 months of age (P517/09/2012)

Refer your communication of February 28, 2014.

This is to acknowledge receipt of the study progress report and hereby grant you annual extension of approval for ethical research Protocol **P517/09/2012.**

The renewal periods are 17th April 2014 – 16th April 2015.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN- ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.

g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN -ERC website **www.uonbi.ac.ke/activities/KNHUoN**

Kindly forward the informed consent documents for endorsement with updated stamp.

Yours sincerely

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. Prof. A.N.Guantai,Chairperson, KNH/UoN-ERC
The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
The Director, UNITID, UoN

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Ref: KNH-ERC/ MOD/502

4th December 2014

Oiye Shadrack Okoth
Principal Investigator
UNITID
College of Health Sciences
University of Nairobi

Dear Dr.Okoth

Re: Approval of modifications – study titled "Effect of maternal HIV status on breast milk intake and growth of HIV-uninfected Kenyan infants at 6 week postpartum and 6 months of age (P517/09/2012)

Your communication of 13th October 2014 refers.

The KNH/UoN-ERC has reviewed and approved change of protocol from HIV testing of infants at 6 months after birth to 9 months after birth. This is acceptable.

Yours sincerely

PROF. M'L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. Prof.A.N. Guantai, Chairperson, KNH/UoN-ERC
The Deputy Director CS
The Principal, College of Health Sciences, UoN

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link:www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/ R/17

17th February, 2015

Oiye Shadrack Okoth
UNITID
College of Health Sciences
University of Nairobi

Dear Shadrack

Re: Approval of annual study renewal –Effect of maternal HIV status on breast mild intake and growth of HIV-uninfected Kenyan infants at 6 week postpartum and 6 months of age ((517/09/2012)

Your communication of 13th February, 2015 refers.

This is to acknowledge receipt of the study progress report and hereby grant you annual extension of approval for ethical research protocol P517/09/2012.

The study renewal dates are 17th February, 2015 to 16th February, 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN- ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.

Annex 6.2: NACOSTI Research Authorization

REPUBLIC OF KENYA

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telephone: 254-020-2213471, 2241349, 254-020-2673550
Mobile: 0713 788 787 , 0735 404 245
Fax: 254-020-2213215
When replying please quote
secretary@ncst.go.ke

P.O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref: **NCST/RCD/12A/013/128**

Date: **20th August, 2013**

Shadrack Okoth Oiye
University of Nairobi
P.O.Box 30197-00100
Nairobi.

RE: RESEARCH AUTHORIZATION

Following your application dated *1st August, 2013* for authority to carry out research on "***Effects of maternal HIV Status on breast milk intake and growth of HIV-Uninfected Kenyan infants at 6 Weeks Post-Partum and 6 Months of age,***" I am pleased to inform you that you have been authorized to undertake research in **Siaya District** for a period ending **31st December, 2015.**

You are advised to report to **the District Commissioner, the District Education Officer and the District Medical Officer of Health, Siaya District** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

DR. M. K. RUGUTT, PhD, HSC.
DEPUTY COUNCIL SECRETARY

Copy to:

The District Commissioner
The District Education Officer
The District Medical Officer of Health
Siaya District.

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THIS IS TO CERTIFY THAT:
Prof./Dr./Mr./Mrs./Miss/Institution
Shadrack Okoth Oiye
of (Address) University of Nairobi
P.O Box 30197-00100, Nairobi.
has been permitted to conduct research in

Siaya	Location
Nyanza	District
	Province

on the topic: Effect of maternal HIV status on breast milk intake and growth of HIV-Uninfected Kenyan at 6 weeks post-partum and 6 months of age.

for a period ending: 31st December, 2015.

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Research Permit No. *NCST/RCD/12A/013/128*
Date of issue *20th August, 2013*
Fee received *KSH. 2000*

Applicant's Signature

For Secretary
National Council for Science &Technology

CONDITIONS

1. **You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit**
2. **Government Officers will not be interviewed without prior appointment.**
3. **No questionnaire will be used unless it has been approved.**
4. **Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.**
5. **You are required to submit at least two(2) hard copies and one(1) soft copy of your final report.**
6. **The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice**

REPUBLIC OF KENYA

NACOSTI

National Commission for Science, Technology and Innovation

RESEARCH CLEARANCE PERMIT

Serial No. A

CONDITIONS: see back page

Annex 6.3: Siaya County Research Approvals

OFFICE OF THE PRESIDENT
MINISTRY OF INTERIOR AND CO-ORDINATION OF NATIONAL GOVERNMENT

Deputy County Commissioner
Siaya Sub County
P O Box 83
SIAYA

Telephone (057) 321770
When replying Please quote
Email address:dcsiaya@yahoo.com

ED.12/12 VOL.II/82

8th October 2013

Mr. Shadrack Okoth Oiye
University of Nairobi
P O Box 30197-00100
NAIROBI

RE: RESEARCH AUTHORIZATION

Reference is made to a letter No. NCST/RCD/12A/013/128 of 20th August 2013 from the Deputy Council Secretary, National Council for Science and Technology on the above subject matter.

Kindly note that you have been granted authority to conduct research on ***" Effects of maternal HIV status on breast milk intake and growth of HIV-Uninfected Kenyan infants at 6 weeks Post-Partum and 6 months of age",*** within Siaya sub county for a period ending 31st December 2013.

Olwande

NOAH O. OLWANDE
FOR: DEPUTY COUNTY COMMISSIONER
SIAYA SUB COUNTY

Cc: The District Education Officer-SIAYA

The Medical Officer of Health –SIAYA

Assistant County Commissioner-Siaya Sub County.

REPUBLIC OF KENYA

MINISTRY OF EDUCATION, SCIENCE & TECHNOLOGY

State Department of Education

Telephone:
Fax:

DISTRICT EDUCATION OFFICER
SIAYA SUB COUNTY
P.O. BOX 199
SIAYA

When replying please quote:
REF: SD/13

DATE: 26th September, 2013

TO WHOM IT MAY CONCERN

RE: RESEARCH AUTHORIZATION – MR. SHADRACK OKOTH OIYE

The above mentioned is a PHD student at the University of Nairobi Institute of Tropical and Infectious Diseases (UNITID) as introduced to this office by office of the Director (UNITID) vide a letter Ref. No. 31350/2012 dated 11th September, 2013.

He is undertaking a research in Siaya District for a period ending 31st December, 2015 as authorized by NCST vide a letter Ref. No. NCST/RCD/12A/013/128 dated 20th August, 2013. The research title is "Effects of maternal HIV status on breast milk intake and growth of HIV-uninfected Kenyan infants at 6 weeks post-partum and 6 months of age".

Kindly, accord him the necessary assistance to enable him complete his research in good time.

DISTRICT EDUCATION OFFICER
SIAYA DISTRICT

SANDE ANDREW
FOR: DISTRICT EDUCATION OFFICER
SIAYA DISTRICT

Annex 6.4: Siaya District Hospital Research Approval

MINISTRY OF HEALTH

Telegrams: "MEDICAL , Siaya
Telephone: Siaya 057-321031
E-mail Siayamoh@yahoo. com
When replying please quote
Ref. No IRC .0014/13

MEDICAL SUPERINTENDENT OFFICE
SIAYA DISTRICT HOSPITAL
P.O. BOX 144
SIAYA.
8TH October, 2013

TO

SHADRACK OIYE OKOTH
UNITID
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF NAIROBI

Dear Mr. Oiye

RE: RESEARCH PROPOSAL

Following your application for authority to carry out research on "Effects of Maternal HIV status on breast milk intake and growth of HIV uninfected Kenyan infants at 6 weeks postpartum and at 6 Months of age" I am pleased to inform you that the IRC sitting on 2nd October 2013 approved your research in Siaya District Hospital. On completion, you are expected to submit soft and hard copies of the findings to our hospital.

Sincerely,

DR. JAMES WAGUDE
CHAIRPERSON INSTITUTIONAL REVIEW COMMITTEE
SIAYA DISTRICT HOSPITAL

Cc.

MEDICAL SUPERINTENDENT