APPLYING A MOTHER-INFANT DYAD PERSPECTIVE TO EXAMINE THE NUTRITIONAL INTERRELATIONSHIPS OF HIV-INFECTED MALAWIAN MOTHERS AND THEIR EXCLUSIVELY BREASTFED INFANTS

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Abstract

ELIZABETH MARIE WIDEN: Applying a mother-infant dyad perspective to examine the nutritional interrelationships of HIV-infected Malawian women and their exclusively breastfed infants
(Under the direction of Linda Adair)

We used data from a large clinical trial, the Breastfeeding, Antiretrovirals and Nutrition (BAN) Study (www.thebanstudy.org), and a nutrition sub-study of BAN, the Malawi Mothers and Infants (MaMi) Study. The BAN study was a large clinical trial that aimed to promote maternal and infant health in HIV-infected Malawian women and their infants, and to prevent maternal to child transmission of HIV by providing a lipid based nutrient supplement (LNS) to the mother and antiretroviral drugs to the mother or infant. Mother-infant pairs (n=2,369) were randomized using a two-arm nutritional and three-arm drug to six study arms: maternal LNS/maternal ARV (mLNS-mARV), maternal LNS/infant ARV (mLNS-iARV), maternal LNS (mLNS), maternal ARV (mARV), infant ARV (iARV), or control (C). The data used in this analysis was derived from screening visits and numerous visits from birth to 24 weeks postpartum. This study provided a novel opportunity to understand how maternal nutritional status was related to infant status, and how infant status related to maternal status. We chose to examine two important aspects of maternal and infant nutritional status during this time: iron and anthropometry. We observed that among mothers with low body mass index (BMI) maternal weight loss was related to less infant weight and length gain from birth to six months in girls. In longitudinal models, higher maternal Hb was associated with higher

concurrently measured infant Hb, especially between 6 and 18 wk. In the MaMi substudy, we observed that increases in maternal TfR and Hb were associated with an increase in infant Hb and TfR from initial measurement to 24 weeks. These findings suggest that maternal breastmilk quality may be compromised in thin women who lose weight, adversely affecting infant growth in females. The observed maternal influence in iron models suggests optimizing maternal iron status during pregnancy and lactation is important for reducing risk of infant iron depletion. Together these results highlight that promoting maternal nutritional status benefits not only the mother, but her infant as well.

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List of Abbreviations and Symbols

α – alpha

AGP – alpha-1-acid glycoprotein

AIDS – acquired immune deficiency syndrome

ARV – antiretrovirals

BAN – Breastfeeding, Antiretrovirals and Nutrition Study

BAN intervention arms –

- mLNS-mARV – maternal LNS/maternal ARV
- mLNS-iARV – maternal LNS/infant ARV
- mLNS – maternal LNS
- mARV – maternal ARV
- iARV – infant ARV
- C – control

BMI – body mass index (kg/m^2)

CD4 – cluster of differentiation – used to define stage of HIV or AIDS infection

CRP – C-reactive protein

Hb – hemoglobin

HIV – human immunodeficiency virus

HIV-EU – human immunodeficiency virus exposed, uninfected

LAZ – length-for-age Z-score

LNS – lipid-based nutrient supplement

MaMi – Malawi Mothers and Infants Study

Stunting – Length-for-age < -2 standard deviations below WHO reference median

TfR – soluble transferrin receptor

WAZ – weight-for-age Z-score

WFP – World Food Program

WHO – World Health Organization

wk- week

WLZ – weight-for-length Z-score

Chapter 1. Introduction

Overview

The mother is the sole source of nutrition for the infant during exclusive breastfeeding, yet there is little known about how maternal and infant nutritional statuses are interrelated and interdependent throughout this time. Though maternal health and nutritional status are important determinants of breastmilk quality and quantity, maternal nutritional status during lactation has primarily been examined as a predictor of child nutritional outcomes; rarely have the effects of infant nutritional status on maternal nutritional status been explored. As we are interested in the health and nutritional status of the mother-infant dyad, we framed our analyses to explore the interrelationships between maternal and child nutrition statuses, to understand the balance between infant nutrient demand and maternal nutritional supply during lactation, and to determine how this relates to infant and maternal anthropometry and iron status.

We developed novel methods to explore the intricate interplay and dynamics between the mother and infant during this time period. In resource poor settings with high HIV prevalence, these relationships may be especially important for long-term maternal and child survival, health, and nutritional status. There currently is a dearth of literature on the nutritional statuses of HIV-infected mothers and their HIV-exposed infants during breastfeeding. These mothers may be unable to simultaneously meet their own and their infant's nutritional needs which could adversely affect the long term health of the mother-infant dyad and may increase susceptibility to pathogens and mother to child transmission of

HIV. Therefore our research provides urgently needed evidence regarding how maternal and infant nutritional statues are interrelated and interdependent between HIV-infected women and their infants in resource poor settings.

Overall objectives and specific aims.

This study focused on the relationships between maternal and infant nutritional status in HIV-infected lactating women and their exclusively breastfed HIV-exposed, uninfected (HIV-EU) infants. We used data from the Breastfeeding, Antiretrovirals and Nutrition Study (BAN), a randomized controlled trial of 2,369 Malawian HIV infected women and their infants to prevent HIV transmission during exclusive breastfeeding conducted between March 2004 and January 2010 in Lilongwe, Malawi, as well as a sub-study of BAN, the Malawi Mothers and Infants Study (MaMi).[1] BAN is unique as it has parallel longitudinal maternal and infant measures of nutritional status, including anthropometry and micronutrient status, during exclusive breastfeeding. We focused on the HIV exposed, uninfected infants and their mothers in BAN, who were followed during pregnancy, birth and 1, 2, 4, 6, 8, 12, 18, 21 and 24 weeks postpartum.[1] The overall goal of this study was to determine the interrelationships of maternal and infant nutrition status during exclusive breastfeeding. Specific aims for this study are as follows:

Aim 1: Determine the interrelationships between maternal and infant anthropometry during exclusive breastfeeding in HIV-infected mothers and their infants.

First, we developed models to determine how maternal weight loss between 2 and 24 weeks postpartum influenced infant growth. We hypothesized that infants of mothers losing weight during lactation will have suboptimal growth.

Aim 2: Determine how maternal iron status during lactation influences infant iron

status during exclusive breastfeeding in HIV-infected mothers and their infants.

First, we developed longitudinal models to determine how maternal hemoglobin (Hb) was associated with concurrently measured infant Hb from 2 to 24 weeks. Then we developed models to determine the association between maternal and infant transferrin receptors (TfR), Ferritin and Hb at 2 and 24 weeks postpartum in the MaMi subsample of mother-infant pairs after accounting for inflammation and infection. We hypothesized that infant Hb at birth and maternal Hb during lactation will influence infant Hb longitudinally, and that effects of maternal Hb will be more marked approaching the 24^{th} week of exclusive breastfeeding when infant iron stores are more likely to be depleted. We also hypothesized that maternal Hb, TfR and Ferritin will predict infant values in the MaMi sub-sample of mother-infant pairs.

Chapter 2. Literature Review

The mother infant dyad

The mother-infant dyad is a conceptual construct to define the connections between the mother and her infant. Previous research examining the mother-infant dyad has focused particularly on mother-infant interactions and attachment during breastfeeding and infancy.[2,3] The dyad has also been studied with regard to evolutionary adaptations to the composition of breast milk.[4,5] Numerous authors have indicated that breastmilk production is focused not only on the survival of the infant, but also on the survival of the mother-infant dyad;[4] suggesting that "maximum evolutionary gain is obtained when protein and energy levels in breastmilk are just high enough to prevent prohibitive infant mortality rates, but low enough to spare the mother."[5] Although we did not test this hypothesis, it provided a framework for our analyses, as we included the health and nutritional status of the mother as a critical component of breastfeeding as well as infant health and survival.

Why anthropometry and iron?

These are two major markers of maternal and child health and nutritional status. Maternal and infant anthropometry measures reflect short and long-term energy balance and overall body composition; providing a general understanding of overall nutritional status, which can easily be translated to public health recommendations. As HIV-infected women have increased energy needs, determining if and when maternal anthropometry changes

influence infant anthropometry and conversely if and when infant growth influences maternal anthropometry is even more important in the context of HIV.

As iron deficiency is a major public health problem affecting 42% of pregnant women and almost half of preschool aged children globally,[6] it is vital to understand if and when maternal and infant iron statuses are related during exclusive breastfeeding. In the context of HIV, iron deficiency is common among HIV-infected women; as such their infants are at heightened risk of iron deficiency. Elucidating this relationship is essential as iron is critical for growth, brain development and immune function.[7] Both iron status and anthropometry are commonly measured in public health settings; therefore, our findings can be translated into public health screening and interventions that can be broadly implemented.

Methods to evaluate the maternal supply and infant demand relationships have not been developed

The relationship between maternal weight changes and infant growth is complicated by the feedback loop between infant breastmilk demand and maternal breastmilk supply (Figure 2.1). When infant demand increases, maternal breastmilk production will increase, subsequently affecting maternal nutritional status. With insufficient dietary intake to meet the energy costs of lactation and also due to hormonal influences, maternal fat stores may be mobilized, and weight changes may result.[8] Increased infant milk intake will subsequently influence infant growth and may lead to additional increases in infant demand and added burden on maternal nutritional status, as a rapidly growing infant is likely to demand more milk.[8] Given adequate nutrient stores and dietary intake, most women may be able to meet infant breastmilk demand; however, if the mother is undernourished, and has inadequate dietary intake or energy reserves, breastmilk energy output may decrease,[9] adversely affecting infant growth. Meeting infant demand may be particularly challenging for HIV

infected mothers, as energy needs are already elevated due to HIV infection; thus examining how maternal supply relates to infant demand is even more imperative in the context of HIV.

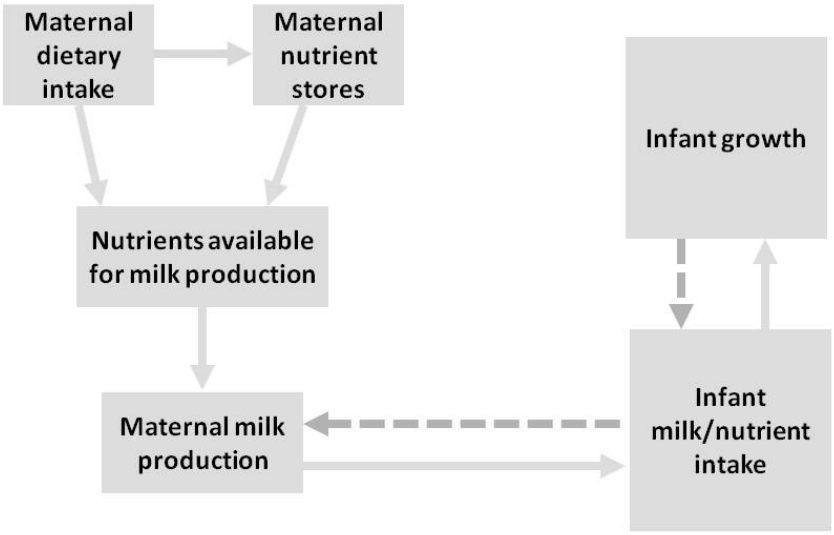

Figure 2.1 Maternal supply and infant demand

The dashed arrows indicate where infant demand may exert influence on maternal nutritional status.Image adapted from "Influence of maternal nutrition on lactation" by Kathy Rasmussen.[8]

Previous analyses utilizing a supply-demand model have tested the fetal origins hypothesis by examining how nutrient restriction during pregnancy relates to markers of cardiovascular disease risk.[10,11] Because these prior analyses focused only on maternal exposures during pregnancy, feedback loops did not complicate these analyses. Accounting for maternal supply and infant demand when evaluating nutritional status during exclusive breastfeeding is complex, as feedback loops cannot be incorporated into linear models. To overcome the constraints of standard regression models, modeling approaches that allow for

incorporation of feedback loops and for testing of complex causal relationships are urgently needed; but difficult to implement.

Maternal anthropometry patterns are highly variable during lactation and are not well understood in the context of HIV

During the first six months postpartum, most lactating women experience mild and gradual weight loss.[12] Among well-nourished women, breastfeeding is usually associated with earlier return to pre-pregnancy weight.[13,14] Among undernourished women, though, weight loss, weight maintenance and even moderate weight gain have been reported.[15-18] Anthropometry patterns during lactation have been less well described in HIV infected women. Similar to HIV uninfected women, though, the findings are inconsistent with studies reporting both gains and losses during lactation.[13]

Maternal nutritional status and lactation performance

Studies have shown that breastfeeding can deplete energy and nutrient reserves of women with inadequate dietary intake and poor maternal nutrition can influence breastmilk quantity and nutrient quality.[8,18,19] Brown et al (1986) examined the lactational capacity of marginally nourished mothers of Bangladeshi infants by examining relationships between maternal nutritional status and breastmilk quantity and quality.[20] In mothers (n=60) with infants who were 90 days old, an association was found between the change in maternal weight from baseline and the quantity of milk, as well as nitrogen and energy content of milk.[20] Furthermore, women who gained at least 200 grams from baseline produced more breastmilk with higher amounts of nitrogen and energy regardless of initial maternal weight.[20] On the contrary, increasing maternal arm circumference was negatively associated with quantity of milk produced; yet this was speculated to result from multicollinearity between maternal arm circumference and weight (R=0.70).[20] Furthermore, poor maternal

nutritional status did not severely impair lactational capacity; though, it limited breastmilk quantity and energy concentration compared to more well nourished mothers.[20]

How maternal HIV infection influences breastmilk supply and lactation performance is not well documented. Prior studies regarding lactation of HIV infected women have focused on issues related to HIV transmission including breastmilk viral load and micronutrient content after heat treatment,[21,22] rather than the relation of maternal nutritional status to the nutrient content and quantity of breastmilk.

Anthropometry patterns of infants during exclusive breastfeeding

After birth infants typically lose weight in the first few days of life, and then return to birth weight by the seventh to tenth day of life.[23] Subsequent growth proceeds at a "rapid but decelerating rate."[23] Birthweight is usually doubled by four to six months of age, and tripled by one year of age.[23] Among well-nourished infants, length is increased by 50% in the first year, and is doubled by four years of age.[23]

It is well established that anthropometry patterns of healthy breastfed infants are different than artificially fed infants.[24] Moreover, the growth patterns of breastfed HIV exposed infants are similar to HIV unexposed infants.[25] In less developed countries, most studies have indicated a lack of association between HIV exposure and postnatal growth parameters including length-for-age, weight-for-age, and weight-for-length compared to HIV unexposed infants.[25] Though there are some studies of the growth patterns of HIV-EU, most of these studies had limited numbers of HIV exposed children and had few anthropometry measurements from birth to 6 months.[25] More evidence is needed to understand infant growth patterns and predictors in HIV exposed infants during this time period.

Few studies have examined how maternal weight changes translate into infant weight and length gain with a longitudinal design

In the East Java Pregnancy Study, increasing maternal post-partum body mass index (BMI) (kg/m^2), measured between 4-6 months, was associated with higher infant weight and length gains between birth and 6 months.[26] Interestingly, infants with lower birthweight had higher length and weight gains compared to infants with higher birthweight; indicating that the breastmilk quality and quantity was sufficient to promote growth of smaller infants, but the growth of infants with higher nutritional needs was compromised.[26] Most of the infants in another study in this population were partially breastfed starting within the first month of life; thus partial breastfeeding (and subsequent exposure to pathogens and other foods) may have biased the effect of post-partum BMI on weight and length gain in this analysis.[26] In Bolivian mother-infant pairs, weight and height at 3 months postpartum were positively correlated with infant weight gain between 3 and 6 months.[27] These findings suggest associations between maternal and infant anthropometry; however, maternal body composition during early lactation and potential confounding variables such as maternal education, parity and morbidity were not accounted for in the models. Moreover, as exclusive breastfeeding may not have continued through the duration of these prior studies, the mother may no longer be the sole source of nutrition for her infant; thus effect estimates may be biased.

The effects of maternal supplementation on infant growth

Several studies have examined the effects of maternal supplementation during pregnancy and lactation on infant growth including Institute of Nutrition of Central America and Panama Oriente longitudinal study in Guatemala and The Bacon Chow Study in Taiwan. In the Oriente longitudinal study conducted between 1969 and 1977, the effects of maternal supplementation during pregnancy and lactation on infant growth were examined.[28] Mothers

were supplemented with a protein-calorie supplement ('atole', gruel typically made with corn) or caloric supplement ('fresco', cool drink).[28] Though not significant, maternal supplementation had a positive effect ($0.05 < p < 0.10$) on infant weight gain from birth to 6 months.[28] Moreover, infants were also supplemented with atole or fresco, thus the effect of maternal supplementation on infant growth cannot be isolated from the effect of infant supplementation.[28] Infant supplementation before 3 months had negative effects on infant weight gain ($p<0.001$), whereas infant supplementation between 3 and 6 months of age was found to positively influence infant growth ($p<0.01$).[28] In the Bacon Chow Study conducted in rural Taiwan starting in 1967 for 6.5 years, the effect of maternal supplementation on infant growth was examined in mothers who gave birth to two infants during course of the study.[29] Mothers were randomized to supplementation with a milk-based formula, providing 800 calories and 40 grams of protein per day, or a placebo, providing 40 calories per day, starting at 15 days after the birth of the first infant and continuing through weaning of the second infant.[29] There were no significant differences between maternal supplementation versus placebo; whereas the timing of maternal supplementation was important, yet inconsistent.[29] Boys were heavier in the second infant cohort ($p=0.02$) compared to the first cohort.[29] Conversely, girls were heavier in the first infant cohort ($p=0.05$) compared to the second.[29]

In the context of HIV, few maternal supplementation studies have been conducted that examine its effect on infant growth. One study in Tanzania found that micronutrient supplementation during and after pregnancy improved child growth.[30] Another study in Tanzania focused on micronutrient supplementation during pregnancy, finding a decreased risk of intrauterine growth restriction with supplementation.[31] Previously we observed that

maternal supplementation with lipid base nutrient supplement (LNS) in BAN participants had no sustained or consistent effects on infant growth from birth to 24 weeks.[32]

By extending our analyses from anthropometry to iron status, we will explore the mother-infant nutritional dyad in two domains

Maternal iron status may influence infant iron status during exclusive breastfeeding; however, many additional factors influence infant iron needs and status. Infant iron stores at birth reflect maternal pregravid and pregnancy iron status, gestational age and birthweight of the infant, and timing of cord clamping.[33] Many factors influence post-natal infant iron status including redistribution of body iron, growth rate, infections and inflammation, and possibly breastmilk iron status.[33] Postnatally, infant Hb exhibits dynamic changes with a peak at birth of approximately 17 g/dL and a nadir of 11.2 g/dL at 2 months of age.[33] Few studies have characterized the iron status of exclusively breastfed HIV-exposed infants.[34]

Most literature suggests maternal iron status strongly affects infant iron status only during pregnancy and has little effect during lactation;[33,35] however, we theorize that in our population of HIV-infected Malawian women, whose iron stores are likely depleted postpartum, maternal iron status may be an important determinant of infant iron status during exclusive breastfeeding. Further investigation of this association is essential, particularly in women who are already at risk of anemia, such as our population of HIV infected women, as their infants may be at heightened risk of iron deficiency.[36,37]

Why Malawi?

Malawi provides a unique opportunity to study the interrelations of maternal and infant nutritional status in a resource poor setting in the context of HIV. When the BAN study began in 2004, almost all Malawian infants were breastfed for some time, with a median duration of breastfeeding of 23.2 months.[38] The median duration of exclusive

breastfeeding was 2.5 months, while the median duration of predominant breastfeeding was 4.8 months.[38] Though the WHO recommends six months of exclusive breastfeeding, 37% of infants ages 4-5 months were given complementary foods.[38]

Based on the most recent Demographic and Health Survey Data, HIV prevalence in Malawi among women of child bearing age is 12% with about a quarter of new infections due to mother to child transmission.[38] Similar to other resource poor settings, food security in Malawi exhibits seasonal variation, with lowest food supplies in rainy season and highest supplies during harvest season.[39] Given the poor food environment and staple foods of low nutritional value, maternal and child nutritional status in Malawi is poor. Among children under 5 years of age, stunting prevalence is very high (48%), while about a quarter of children are underweight.[38,40] As almost half of women of childbearing age have iron deficiency anemia, it is not surprising that almost 80% of preschool aged children have iron deficiency anemia.[38] As maternal and child nutrition in Malawi is suboptimal, our findings provide direly needed evidence for interventions and policies targeting the mother-infant dyad and, furthermore, bring the health and nutritional status of mothers into focus.

Several formative studies for BAN were conducted regarding infant feeding and maternal nutrition, providing contextual background for our research. Attitudes of the role of maternal nutrition in breastfeeding among HIV positive women was examined through in-depth interviews with twenty-two HIV positive women living in semirural areas near Lilongwe.[41] Most of the women perceived that larger body shapes were healthier and were aware of the need to maintain a high-quality diet for their own health and to meet the nutritional demands of lactation.[41] Additional formative research for BAN investigated attitudes regarding infant feeding practices.[42] Semi-structured interviews were conducted

with 40 HIV positive mothers. 85% of HIV positive mothers reported giving water or porridge to their infant before 4 months of age.[42] Moreover, 60% of the 17 mothers with undisclosed HIV status had introduced complementary foods before 6 months of age.[42] One mother reported: "Traditionally, we are told that if a child cries it is a sign of hunger. Because my baby was crying, I thought my breast milk was not adequate, so I started giving him water at 3 months."[42] These formative studies guided the development of the infant-feeding counseling protocol for BAN.[43] The implementation of the BAN infant-feeding counseling protocol was evaluated with observations of counseling sessions and interviews with the study nurses in a convenience sample of 123 HIV positive mothers who visited the study clinic between November and December 2005.[43] Through process evaluation of the nurses' adherence to the protocol indicate an average adherence to the protocol of ≥90%.[43]

Chapter 3. Maternal weight loss during exclusive breastfeeding is associated with reduced weight and length gain in daughters of HIV-infected Malawian women

Overview

Maternal weight loss during exclusive breastfeeding may influence growth of exclusively breastfed infants through impaired quality or quantity of breastmilk. This study evaluated how maternal weight loss from 2-24 wk was related to infant weight and length gain in 1,390 lactating HIV-infected mothers and their exclusively breastfed infants. Malawian mother-infant pairs in the Breastfeeding, Antiretrovirals, and Nutrition (BAN) study were randomized to receive a lipid-based nutrient supplement (LNS), meeting nutritional needs of lactation, or no LNS and a maternal, infant or no antiretroviral (ARV) regimen. Linear regression models were used to relate maternal weight loss (weight loss vs. no weight loss) to infant weight and length gain from birth to 24 wk, stratifying by gender, and controlling for infant birthweight/length and maternal BMI at 2 wk (mean: 23.1 kg/m^2) and interacting maternal BMI with weight loss. Length (β: -3.24 cm, p=0.01) and weight gain (β: -1.21 kg, p=0.008) were lower in girls whose mothers had lower BMI at 2 wk postpartum coupled with the weight loss, compared to girls of women who did not lose weight. Though associations were only observed in girls, suggesting possible gender differences in suckling and feeding behavior, these findings indicate that maternal weight loss with low energy reserves represents a risk factor for poor infant growth outcomes.

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Introduction

During exclusive breastfeeding, the mother is the sole source of nutrition for her infant. Breastfeeding can deplete energy and nutrient reserves of women with inadequate dietary intake and very poor maternal nutrition can impair breastmilk quantity and nutrient quality.[8,18-20] Increases in maternal weight during lactation have previously been linked to improved quantity and nutrient quantity of breastmilk in marginally nourished Bangladeshi women;[20] yet, it is not well understood how these weight changes translate into breastmilk output and infant growth due to the complex nature of this relationship.[8]

Maternal breastmilk supply is regulated to match infant demand through frequency and intensity of infant suckling.[8,44] With increased infant demand, maternal breastmilk production will increase. In the absence of adequate dietary intake to meet the energy costs of lactation and due to hormonal influences of lactation, maternal fat stores may be mobilized to meet the demand—with implications for maternal weight changes.[8] Increased milk intake will subsequently influence infant growth and may lead to further rises in infant demand and additional burden on maternal nutritional status, as a rapidly growing infant will likely demand more milk.[8] Given adequate maternal nutrient stores and dietary intake, most women may be able to meet infant breastmilk demand; however, in cases where mothers are undernourished and are losing weight, breastmilk energy output may decrease if the mother exceeds her lactational capacity (the ability to produce milk on demand),[9] which could adversely affect infant growth.

In resource-poor settings with high HIV prevalence, six months of exclusive breastfeeding by HIV infected women is recommended to promote child survival if replacement feedings are not acceptable, feasible, affordable, sustainable and safe.[45] Since

HIV infection and lactation increase metabolic demands,[46] lactating HIV infected women may be unable to simultaneously meet their own and their infant's nutritional needs; thus understanding how maternal weight changes during lactation influence infant growth may be especially important for promoting health and survival of the mother-infant dyad in the context of HIV.

During the first six months postpartum, most lactating women experience mild and gradual weight loss.[12] Even among well-nourished women, breastfeeding is usually associated with earlier return to pre-pregnancy weight, but has also been associated with weight gain and weight maintenance.[13,14] Among undernourished women, weight loss, weight maintenance and even moderate weight gain have been reported.[15-18] Anthropometry patterns during lactation have been less well described in HIV infected women. Similar to HIV uninfected women, though, the findings are inconsistent with studies reporting both weight gains and losses during lactation.[13]

Although few studies have reported how the overall pattern of maternal weight changes during breastfeeding influence infant growth, there is evidence of associations between maternal and infant anthropometry in HIV-uninfected and HIV-infected populations. In Indonesian mother-infant pairs, increasing maternal post-partum body mass index (BMI) (kg/m^2), measured between 4-6 months, was associated with higher infant weight and length gains between birth and 6 months.[26] In Bolivian mother-infant pairs, maternal weight and height at 3 months postpartum were positively correlated with infant weight gain between 3 and 6 months.[27] In thirty-five low income Indian women, a 1 kg loss in maternal fat mass from baseline (measured within 1 month of birth) to 6 months postpartum was associated with 107 gram higher infant weight gain ($p=0.04$) at 6 months,

after controlling for baseline maternal weight, infant birthweight and changes in maternal appendicular skeletal mass.[47] In HIV-infected and uninfected mothers and infants in Kenya ages 4 to 24 mo, higher maternal weight was associated with higher concurrently measured length-for-age (LAZ), weight-for-age (WAZ) and weight-for-length (WLZ) z-score, while higher maternal height was associated with higher LAZ and WAZ.[48] Higher maternal BMI and mid-upper-arm circumference was associated with higher WAZ and WLZ.[48] To better understand the relationship between maternal weight changes and infant growth, it may be important to evaluate how the overall pattern of maternal weight change influences infant growth.

Our objective was to examine the how maternal weight loss from 2 to 24 wk relates to infant growth from 0 to 24 wk, corresponding with exclusive breastfeeding in a large sample of mother-infant pairs participating in the Breastfeeding, Antiretrovirals, and Nutrition (BAN) study. The Malawi Mothers and Infants (MaMi) study is an analysis of anthropometric and nutrition data from the BAN study. Mothers in the BAN study who received lipid based nutrient supplement (LNS) had less weight loss during the exclusive breastfeeding (0-24 wk), than mothers who did not receive LNS regardless of antiretroviral drug assignment.[49] In this paper, we examine how infant weight and length gain are influenced by the net change in maternal weight during lactation. We assume infant growth is a proxy indicator of lactation adequacy.[50] To provide contextual background for our findings, we also examined how the maternal weight loss was influenced by BAN treatment arm, parity, and seasonality. We hypothesized that the effects of maternal weight loss on infant weight and length gain will depend on initial energy stores, with more detrimental effects if

the mother has limited stores to mobilize. We expected to observe similar effects on infant weight gain and linear growth.

Methods

The BAN study design has been described in detailed elsewhere.[51] Briefly, BAN was a randomized controlled trial of 2,369 mother-infant pairs that was conducted from April 2004 to February 2010 in Lilongwe, Malawi. HIV 1-positive pregnant women (n=3572) were recruited from four antenatal clinics. Initial screening criteria included: age ≥ 18 years (or ≥14 years of age if married), CD4 count ≥ 250 cells/μL (prior to 11/13/2006 CD4 cut off was ≥200 cells/μL), hemoglobin (Hb) ≥ 7 g/dL, no prior antiretroviral medication use, and no major pregnancy complications.[1] Eligible women (n=2791) who delivered at the study site were provided with maternal and infant single dose nevirapine peripartum as well as twice-daily zidovudine and lamivudine for 7 days postpartum. Within 36 hours after delivery mother-infant pairs had to present at the study site and meet secondary eligibility criteria for randomization: infant birthweight ≥ 2 kg, no severe congenital malformations, no other conditions incompatible with survival or that would preclude the use of the study drugs. 2382 mother-infant pairs met these criteria and thirteen women declined further participation. 2369 women completed informed consent and were randomized using a permuted-block method to one of six treatment arms according to a two-arm nutritional and three-arm antiretroviral factorial design. Half of the mothers received daily maternal LNS providing estimated added energy and protein requirements of lactation as well as the recommended daily allowance of micronutrients, excluding vitamin A. Mother-infant pairs were further randomized to maternal or infant antiretroviral drug, or no post-natal antiretroviral regimen. Details regarding the LNS and drug regimens are described elsewhere.[49] Randomized infants

diagnosed with HIV-1 within two wk of delivery (n=119) with polymerase chain reaction (PCR) were withdrawn from the study, and mother-infant pairs were referred for care.[1] In June 2006, the BAN study initiated cotrimoxazole preventive therapy for infants 6-36 wk of age, based on WHO recommendations.[52]

To buffer the effects of seasonal food shortages and to prevent sharing of maternal LNS, all participants were given 2 kg of maize per week for family consumption. During a drought from February to August 2005, the World Food Program (WFP) provided food aid to all HIV-infected women in Lilongwe, consisting of a monthly ration of corn/soy flour—similar to the quantity in the BAN maize supplement—and 1 liter of vitamin-A-fortified corn oil. This was provided in lieu of the BAN maize package to an estimated 260 BAN mothers and was evenly distributed across study arms.[32]

In accordance with the World Health Organization prevention of maternal to child transmission of HIV (PMTCT) guidelines when the study was designed, [53] all BAN mothers were provided intensive counseling to exclusively breastfeed their infants for 24 wk and to rapidly wean by 28 wk.[43] Only data up to 24 wk are included in this analysis, corresponding with the period of exclusive breastfeeding.

The Malawi National Health Science Research Committee and the institutional review boards at University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention approved the BAN protocol.

Anthropometrics and study procedures

Study visits and data collection were conducted at the BAN study clinic at Bwaila Hospital in Lilongwe. Mother-infant pairs were followed at birth and 2, 4, 6, 8, 12, 18, 21, and 24 wk postpartum. At all visits, maternal and infant weight was measured to the nearest

0.1 kg unit using Tanita digital electronic scales, which were calibrated regularly. Maternal height was measured with a wall-mounted stadiometer. Infant recumbent length was measured using a wooden length board made to UNICEF specifications.[54] Nurses and nutrition assistants were trained in anthropometrics and their measurements were obtained using standard methods.[54]

Infant HIV status was tested with Amplicor 1.5 DNA PCR at 0, 2, 12 and 28 wk. Postnatal HIV tests were confirmed with a specimen obtained at the following visit. Dried blood spots, collected at all study visits excluding the visit at 21 wk, were tested using Gen-Probe Aptima HIV-1 Qualitative assay to further refine the timing of infection in infants with positive PCR results.

Maternal report of parity, marital status, and years of maternal education was obtained at the screening visit. Maternal report of infant's exclusive breastfeeding status was obtained at 4, 8, 12, 18, 21 and 24 wk postpartum. Maternal report of maternal and infant morbidity occurring prior to the visit was obtained at each visit. Physician report of maternal or infant illness was obtained if the mother reported illnesses.

Malawi has a subtropical climate with four seasons: cool (May to mid-August), hot (mid-August to November), rainy (November to April) and post-rainy (April to May). Availability of food, malnutrition and morbidity due to infectious disease vary by season due to rainfall and agricultural production.[55] Season of poor food security is generally from November/December to March/April. This period is characterized by limited food availability, as crop stores are depleted.

Statistical Analyses

Due to normal physiologic changes in the immediate post-partum period and the various factors that could potentially influence the maternal weight measurement at delivery (edema, timing of measurement relative to delivery), we analyze the pattern of maternal weight change from 2 to 24 wk. For simplicity of interpretation and to test the theory proposed by Brown and Dewey,[9] where women with insufficient energy reserves will have suboptimal milk energy output if they are losing weight,[9] we utilized a dichotomous maternal weight change pattern (weight loss vs. no weight loss) in this analysis. Maternal weight at 2 weeks was subtracted from maternal weight at 24 weeks to determine whether the mother was losing weight (weight change <0) or not losing weight (weight change ≥ 0) during exclusive breastfeeding. Alternate specifications of maternal weight change, as a continuous and categorical variable (tertiles and quartiles of maternal weight change), were examined as potential primary exposure variables. Compared to the categorical weight loss (weight loss vs. no weight loss), continuous maternal weight change and quantile categories (tertiles and quartiles) were observed to have similar model fit and effects.

Infant growth models evaluated the influence of maternal weight loss, controlling for infant initial anthropometric measurement, and maternal body mass index (BMI) at 2 wk postpartum. The interaction term between maternal BMI and maternal weight loss tested whether the effects of maternal weight loss varied by initial maternal energy reserves. Adjusted models also included seasonality, parity, BAN treatment arm, education and maternal CD4 count. Adjusted models also included infant morbidity from birth to 24 weeks including fever, vomiting and diarrhea. Illnesses were modeled as the number of instances of illness over number of visits from 0 to 24 weeks. Infant growth models were gender stratified

given the significant gender differences observed in infant weight and length gain, and a significant three-way interaction between maternal weight loss, maternal BMI and gender in the adjusted infant growth models (Length gain interaction p=0.04; Weight gain interaction p=0.08).

Logistic regression was used to evaluate predictors of maternal weight loss, including seasonality, parity, BAN treatment arm, education and maternal CD4 count. Seasonality was measured as month of birth in order to allow us to estimate the pattern of seasonal effects on maternal and infant anthropometry. Birth month allows us to understand the timing of maternal exposure to food insecurity during pregnancy, as well as food availability to the mother following delivery. October was selected as the referent birth month, as mothers were not exposed to periods of food insecurity during the latter part of pregnancy, but were exposed during breastfeeding. Given no observed differences in the likelihood of maternal weight loss between the months of Sept-Nov (p>0.1), these months were combined together as the reference month for seasonality.

Mother-infant pairs were included in the analysis sample if they had data at 0 and 24 wk for the infant, and 2 and 24 wk for the mother. Multiple births (n=49) were excluded from this analysis, as multiples exhibit different growth patterns than singletons and mothers of multiples have different gestational weight gain and postpartum weight change patterns.[56] Infants who were mixed fed or weaned prior to 24 wk (n=248) were also excluded, as the mother was no longer the sole source of nutrition for her infant. Owing to differences in growth and nutrient dynamics of HIV infected infants, HIV infected infants (n=58) diagnosed after 2 wk postpartum were excluded.[1] A small number (n=6) of implausible infant

weights and lengths at 24 wk were replaced by values interpolated from prior and subsequent measurements. Statistical analyses were conducted with STATA 12.1 (College Station, TX).

Results

Mean weight loss of mothers from 2 to 24 wk was 0.93 kg (SD: 3.4 kg) (Table 3.1). 63.3% of mothers lost weight from 2 to 24 weeks, while 36.7% gained weight or had no weight changes during this period. The proportion of mothers receiving the LNS did not differ between the weight loss and no weight change groups (p=0.85). Of the women that lost weight (n=829), mean weight loss was 2.5 kg. Of the mothers that gained weight (n=469), mean weight gain was 2 kg from 2 to 24 wk.

Maternal weight loss was associated with less length and weight gain in girls, particularly among mothers with lower BMI values at 2 wk (Table 3.2; Figure 3.1). Effects of maternal weight loss were strengthened after adjusting for confounders. Maternal weight loss from 2 to 24 wk had no effects on weight or length gain in boys (p>0.05), even at varying BMI levels in adjusted and unadjusted models (Figure 3.1). In adjusted models, few factors significantly influenced infant weight and length gain from birth to 24 wk (Table 3.2), consistent with previous findings in BAN infants.[32] Higher birth length was associated with decreased length gain in boys and girls. Maternal ARV treatment was associated with lower length gain in boys and girls, while infant ARV treatment was associated with lower length gain only in girls. Maternal LNS was not associated with infant weight or length gain. July birth was associated with greater weight gain in boys and girls. Other than July, only February was associated with increased weight gain, but only in girls (p=0.02). For boys, January births were associated with decreased length gain (p=0.03). August births were associated with increased length gain in girls (p=0.003).

Taller maternal height (p=0.04) and the maternal ARV arm (p<0.001) were associated with increased odds of maternal weight loss, while the LNS intervention, primiparity, low CD4 count, and maternal BMI at 2 wk were not associated with the likelihood of maternal weight loss (Table 3.3). Birth month was significantly associated with maternal weight loss [χ^2 (9) =81.33, p<0.001]. Compared to Sept-Nov, births from January to June were associated with reduced odds of weight loss (all p-values <0.001).

Sensitivity analyses showed that including HIV infected infants who had maternal and infant weight data at birth, 2 wk and 24 wk and were still exclusively breastfed at 24 wk (n=28) did not influence the interpretation of results.

Discussion

In our population, we expected to observe adverse effects of low maternal BMI in both boys and girls; however, lower maternal BMI only adversely affected the growth of girls. Initial maternal BMI was not different by infant gender; but mothers of boys lost marginally more weight than mothers of girls (p=0.07). Given our differential findings by gender, it appears that the milk production of undernourished mothers of girls could have been limited by substrates available for milk biosynthesis or their lactational capacity.[8] Perhaps mothers of boys with lower BMIs were better able to mobilize existing nutrient stores or dietary intake into breastmilk production than mothers of girls; yet we are unable to test this as we do not have macronutrient content of breastmilk. Gender preferences in feeding practices are unlikely to explain our findings, as there is little prior evidence of gender preferences in Malawi.[57]

The observed gender differences in maternal weight loss effects are in contrast to what was expected, as boys gain weight and length more rapidly than girls,[58] which may

enhance their susceptibility to environmental insults, such as suboptimal breastmilk quality and quantity. Boys are more vulnerable to undernutrition in utero, as they have faster growth rates and their placentas are believed to have less reserve capacity compared to girls.[59] As a result of this fetal environment, boys may be programmed to be more adaptive to nutritional insults postpartum, and may have an enhanced ability to demand more milk through suckling and feeding behavior despite suboptimal maternal milk output.

Though previous studies have reported associations between maternal weight change and quantity and energy content of milk,[20] sex differences in milk energy output have not been reported in humans. In rhesus macaques, mothers of males, especially if primiparous produced more energy dense, high fat milk, but produced less milk overall, compared to mothers of females.[60] Though mothers produced similar milk energy amounts between males and females, it is unclear whether reduced quantity but higher energy content adversely affects growth.[60] As higher breastmilk energy and protein consumption has been observed to positively influence infant growth,[20] our observed sex differences could possibly be attributable to sex differences in human milk energy output.

Notably few factors influenced infant weight and length gain in the adjusted models, consistent with previous findings in BAN infants.[32] Since BAN infants were exclusively breastfed, a requirement for inclusion in this analysis, this suggests that previously observed predictors of infant growth such as seasonality and parity are not as influential when the infant is exclusively breastfeeding. Exclusive breastfeeding appears to buffer the adverse effects of seasonality on infant weight and length gain. Infant breastmilk intake was observed to decline during the rainy season in The Gambia, which is when mothers usually lose weight,[61] and was initially attributed to maternal energy restriction.[61] However, dietary

supplementation of lactating women in a subsequent study had no effects on maternal milk volume in The Gambia.[61] Given these findings and additional studies reporting seasonal variations breastmilk intake in Kenya and Zaire, the authors concluded that the observed seasonal variations in breastmilk output were attributed to increased infant morbidity and altered breastfeeding patterns due to strenuous maternal farm work.[61] Since BAN infants were closely followed and treated for illnesses throughout the study, seasonal morbidity effects on breastmilk intake could have been limited. Moreover, mothers were intensively counseled to exclusively breastfeed. As such, their infants were protected by the immunological components of milk and were not exposed to pathogens through contaminated foods and fluids. In adjusted models, most morbidities were not predictors of infant weight or length gain. In the length gain adjusted models, vomiting was associated with diminished length gain only in the boys.

Similar to other studies in HIV infected and non HIV-infected populations, maternal weight change patterns were heterogeneous during exclusive breastfeeding with some mothers losing and others gaining weight.[12,16] While taller stature was associated with the maternal weight loss, the likelihood of weight loss was not related to initial BMI. Primiparity, low CD4 count, marital status and education beyond primary school were not associated with maternal weight loss. Though maternal CD4 count has previously been associated with weight loss in a population of breastfeeding HIV-infected Zambian women,[13] inclusion requirements for BAN (CD4>250 cells/μL) may have reduced our ability to observe negative effects of low maternal CD4. The maternal ARV intervention was associated with weight loss from 2 to 24 wk. One of the drugs in the BAN regimen was lopinavir/ritonavir (Kaletra), which is frequently associated with diarrhea. As such, diarrhea

may have contributed to the weight loss in this group.[6262] Maternal LNS had no association with maternal weight loss from 2 to 24 wk postpartum. Though LNS was found to have a small protective effect on maternal weight loss from 0 to 28 wk in an intent-to-treat analysis of BAN mothers (n=2369) the difference between treatment arms was reported to be of little clinical importance;[49] our lack of LNS findings is not surprising given our much smaller analytic sample (n=1309) and our focus on only the period of exclusive breastfeeding from 2 to 24 wk. Mothers who gave birth from January through June were protected from being in the maternal weight loss group. These women were exposed to food insecurity during pregnancy and in the postpartum period, but were not as exposed to food insecurity for the duration of their postpartum weight changes, compared to mothers of infants born between Sept and November. Though many factors influenced the odds of maternal weight loss, few of these factors affected infant weight and length gain; which indicates that maternal breastmilk output was sufficient to mitigate the effects of these factors, even though the mother was losing weight.

This research has some limitations. First, we only had available data on maternal and infant weight and length, and did not have detailed information on body composition, which would have allowed us to understand how changes in maternal body composition related to infant growth and body composition. Effects of maternal weight change on infant growth are likely mediated through breastmilk quantity and quality, which was not measured in this study. Second, we excluded about 45% of randomized mother-infant pairs from our analysis who were lost to follow-up (n=620), multiple births (n=49), weaned early (n=248) or HIV infected infant in first 2 wk (n=199) or HIV-infected infant from 2 to 24 wk (n=58). Compared to randomized infants who were not included in the analysis sample (n=1060),

infants included in our analysis (n=1309) had higher birthweight (p=0.005), lower prevalence of low birth weight (p=0.009), higher birth length (p=0.002) and were more likely to receive the ARV intervention (p=0.006); however, there were no differences between included and excluded infants in infant gender (p=0.14) or Hb at birth (p=0.96). As worse off infants were lost-to-follow up or excluded, we may have underestimated the effects of maternal weight changes on infant growth.

This study also has many strengths. Applying a dyadic modeling framework is an innovative approach to examine these mother-infant anthropometry relationships. Supporting this analysis approach are the detailed exclusive breastfeeding data, which allowed us to exclude weaned and mixed fed infants from our analyses, as the mother was no longer the sole source of nutrition for her growing infant. Moreover, our analysis focused on the period of the most rapid infant weight and length gain and highest maternal energy demands, enhancing our ability to observe effects of maternal weight loss. Finally, understanding these relationships is especially important in the context of HIV in order to guide future interventions and programs to promote maternal and infant health.

In conclusion, our findings suggest that maternal weight loss coupled with low energy reserves may adversely affect infant growth. Further research to understand this relationship is indicated, especially to determine why effects were only seen in girls.

Table 3.1 Characteristics of 1,309 BAN mother-infant pairs in the primary analysis of the effects of maternal weight loss on infant growth

MOTHERS	
Age (y)[1]	26.7 ± 5.1
Height (cm)[1]	156.9 ± 5.5
Weight (kg)[1]	56.2 ± 8.8
Maternal body mass index (kg/m^2)[1]	22.8 ± 3.2
Primiparous (%)	9.6
Married (%)	92.3
Education beyond primary school (%)	36.1
Hemoglobin (g/dL) [1,2]	10.9 ± 1.2
CD4 count (cells/µL)[1,2]	475.4 ± 195.7
CD4 <350 cells/µL (%)[2]	29.9
Received ARV (%)	35.8
Received LNS supplement (%)	51.8
Maternal weight change from 2 to 24 wk(kg)[1]	-0.93 ± 3.4
INFANTS	
Male gender (%)	51.5
Birth weight (kg)[1]	3.04 ± 0.40
Low birth weight (%)[3]	6.0
Birth length (cm)[1]	48.3 ± 1.9
Hemoglobin at birth (g/dL)[1]	17.4 ± 2.01
Received ARV (%)	38.4
Weight gain from birth to 24 wk (kg)[1]	4.13 ± 0.82
Length gain from birth to 24 wk (cm)[1]	15.7 ± 2.2

[1]Mean +/- SD (all such values)
[2] Measured during pregnancy
[3]Low birth weight 2-2.5 kg as infants had to weigh ≥2.0 kg to be eligible for BAN
BAN, Breastfeeding Antiretoviral and Nutrition; LNS, lipid-based nutrient supplement; ARV, antiretroviral intervention.

Table 3.2 Gender stratified linear regression models showing the effects of maternal weight loss on infant weight and length gain from 0 to 24 wk

	Weight gain (kg)						Length gain (cm)					
		Crude			Adjusted			Crude			Adjusted	
	Coef	95% CI	p-value	Coef	95% CI	p-value	Coef	95% CI	p-value	Coef	95% CI	p-value
BOYS		n=673			n=653			n=667			n=647	
Birth measurement	-0.20	[-0.35,-0.04]	0.01	-0.15	[-0.31,0.01]	0.06	-0.42	[-0.51,-0.34]	<0.001	-0.43	[-0.51,-0.35]	<0.001
Mom lost weight	-0.10	[-1.12,0.92]	0.84	-0.09	[-1.14,0.97]	0.87	1.30	[-1.30,3.90]	0.33	1.50	[-1.15,4.14]	0.27
Maternal BMI at 2 wk	0.03	[-0.003,0.07]	0.08	0.02	[-0.02,0.06]	0.27	0.07	[-0.02,0.16]	0.13	0.06	[-0.03,0.15]	0.20
Mom lost *Mom BMI	0.01	[-0.04,0.05]	0.79	0.01	[-0.04,0.05]	0.82	-0.06	[-0.17,0.05]	0.30	-0.07	[-0.18,0.05]	0.26
CD4 <350				0.06	[-0.08,0.20]	0.39				0.12	[-0.24,0.47]	0.52
Fever				-0.03	[-0.10,0.04]	0.37				0.16	[-0.004,0.33]	0.06
Vomiting				-0.01	[-0.15,0.12]	0.83				-0.38	[-0.72,-0.05]	0.03
Diarrhea				-0.05	[-0.17,0.06]	0.38				0.15	[-0.14,0.45]	0.31
Primiparous				0.01	[-0.22,0.24]	0.91				-0.25	[-0.82,0.32]	0.39
Birthmonth (ref: Sept-Nov)												
January				-0.12	[-0.38,0.14]	0.36				-0.76	[-1.40,-0.11]	0.02
February				0.05	[-0.20,0.30]	0.71				-0.22	[-0.85,0.41]	0.49
March				0.22	[-0.04,0.48]	0.10				0.17	[-0.49,0.82]	0.61
April				-0.03	[-0.29,0.23]	0.80				-0.21	[-0.87,0.45]	0.54
May				0.01	[-0.22,0.25]	0.92				0.17	[-0.42,0.77]	0.57
June				-0.11	[-0.36,0.14]	0.40				-0.26	[-0.89,0.37]	0.42
July				0.30	[0.06,0.55]	0.02				0.30	[-0.33,0.92]	0.35
August				0.17	[-0.09,0.42]	0.20				0.50	[-0.15,1.15]	0.13
Dec				0.03	[-0.22,0.28]	0.80				-0.20	[-0.82,0.43]	0.54
Married				0.17	[-0.06,0.39]	0.15				0.12	[-0.46,0.69]	0.69
Education				0.0005	[-0.13,0.13]	0.99				0.31	[-0.02,0.64]	0.07
Mom ARV				-0.13	[-0.29,0.04]	0.12				-0.59	[-1.00,-0.18]	0.01
Mom LNS				0.07	[-0.06,0.19]	0.29				-0.03	[-0.35,0.29]	0.88
Kid ARV				-0.07	[-0.23,0.09]	0.42				-0.25	[-0.65,0.15]	0.22
Intercept	4.16	[3.29,5.02]	<0.001	4.16	[3.23,5.09]	<0.001	35.04	[30.75,39.34]	<0.001	35.48	[31.04,39.91]	<0.001
GIRLS		n=633			n=609			n=632			n=608	
Birth measurement	-0.26	[-0.43,-0.10]	0.002	-0.30	[-0.47,-0.13]	0.001	-0.46	[-0.55,-0.37]	<0.001	-0.46	[-0.56,-0.36]	<0.001
Mom lost weight	-0.97	[-1.86,-0.08]	0.03	-1.29	[-2.20,-0.38]	0.01	-2.38	[-4.83,0.08]	0.06	-3.36	[-5.88,-0.85]	0.01
Maternal BMI at 2 wk	0.03	[-0.001,0.06]	0.06	0.02	[-0.01,0.05]	0.12	-0.005	[-0.08,0.07]	0.90	-0.03	[-0.11,0.05]	0.49
Mom lost *Mom BMI	0.04	[0.004,0.08]	0.03	0.06	[0.02,0.09]	0.01	0.1	[-0.01,0.20]	0.07	0.14	[0.03,0.25]	0.01
CD4 <350				0.03	[-0.10,0.16]	0.70				-0.19	[-0.55,0.17]	0.31
Fever				0.01	[-0.06,0.07]	0.84				0.11	[-0.07,0.28]	0.23
Vomiting				-0.0005	[-0.13,0.13]	1.00				-0.01	[-0.38,0.37]	0.98
Diarrhea				-0.03	[-0.16,0.10]	0.63				-0.003	[-0.36,0.35]	0.99

Primiparous				-0.13	[-0.34,0.07]	0.20				0.21	[-0.35,0.76]	0.46
Birthmonth (ref: Sept-Nov)												
January				0.04	[-0.21,0.28]	0.78				0.04	[-0.66,0.73]	0.92
February				0.32	[0.05,0.59]	0				0.66	[-0.09,1.41]	0.08
March				-0.02	[-0.28,0.24]	0				-0.32	[-1.05,0.41]	0.39
April				0.14	[-0.11,0.39]	0				-0.08	[-0.78,0.61]	0.82
May				0.09	[-0.14,0.32]	0.43				0.35	[-0.28,0.98]	0.27
June				0.17	[-0.06,0.39]	0.14				0.64	[0.02,1.26]	0.04
July				0.42	[0.17,0.66]	0.001				0.16	[-0.52,0.84]	0.64
August				0.13	[-0.09,0.35]	0.25				0.88	[0.28,1.49]	0.004
Dec				0.23	[-0.04,0.49]	0.09				0.21	[-0.52,0.94]	0.57
Married				-0.05	[-0.29,0.19]	0.70				0.10	[-0.56,0.76]	0.77
Education				0.05	[-0.08,0.18]	0.42				0.16	[-0.20,0.52]	0.38
Mom ARV				-0.04	[-0.20,0.11]	0.58				-0.43	[-0.87,0.02]	0.06
Mom LNS				0.08	[-0.05,0.20]	0.22				0.21	[-0.12,0.55]	0.21
Kid ARV				-0.11	[-0.26,0.05]	0.18				-0.58	[-1.01,-0.15]	0.01
Intercept	4.06	[3.32,4.80]	<0.001	4.22	[3.42,5.01]	<0.001	37.64	[33.07,42.21]	<0.001	38.03	[33.21,42.86]	<0.001

BAN, Breastfeeding Antiretoviral and Nutrition; LNS, lipid-based nutrient supplement; RV, antiretroviral intervention

Table 3.3 Predictors of maternal weight loss among BAN mothers

	Maternal weight loss from 2-24 wk[1]		
	OR	95% CI	p
BMI at 2 weeks postpartum	1.02	[-0.02,0.06]	0.32
Maternal height (cm)	1.02	[0.00,0.04]	0.04
CD4 <350 cells/µL	1.14	[-0.13,0.40]	0.33
Primiparous	1.01	[-0.41,0.43]	0.96
Birthmonth (ref: Sept, Oct, Nov)[2]			
January	0.35	[-1.52,-0.56]	<0.001
February	0.32	[-1.62,-0.64]	<0.001
March	0.19	[-2.16,-1.17]	<0.001
April	0.29	[-1.74,-0.77]	<0.001
May	0.35	[-1.51,-0.62]	<0.001
June	0.42	[-1.34,-0.41]	<0.001
July	1.05	[-0.48,0.59]	0.85
August	0.77	[-0.75,0.23]	0.30
December	0.62	[-0.99,0.03]	0.07
Married	1.49	[-0.05,0.84]	0.08
Education	1.22	[-0.06,0.46]	0.13
Mom ARV	1.74	[0.29,0.81]	<0.001
Mom LNS	0.98	[-0.26,0.22]	0.87
Intercept	0.03	[-7.11,0.11]	0.06

[1]Maternal weight loss from 2-24 wk, compared to weight change ≥0

[2]Chunk test for season effects: chi2(9) 81.33, p<0.001

BAN, Breastfeeding Antiretoviral and Nutrition; LNS, lipid-based nutrient supplement; ARV, antiretroviral intervention.

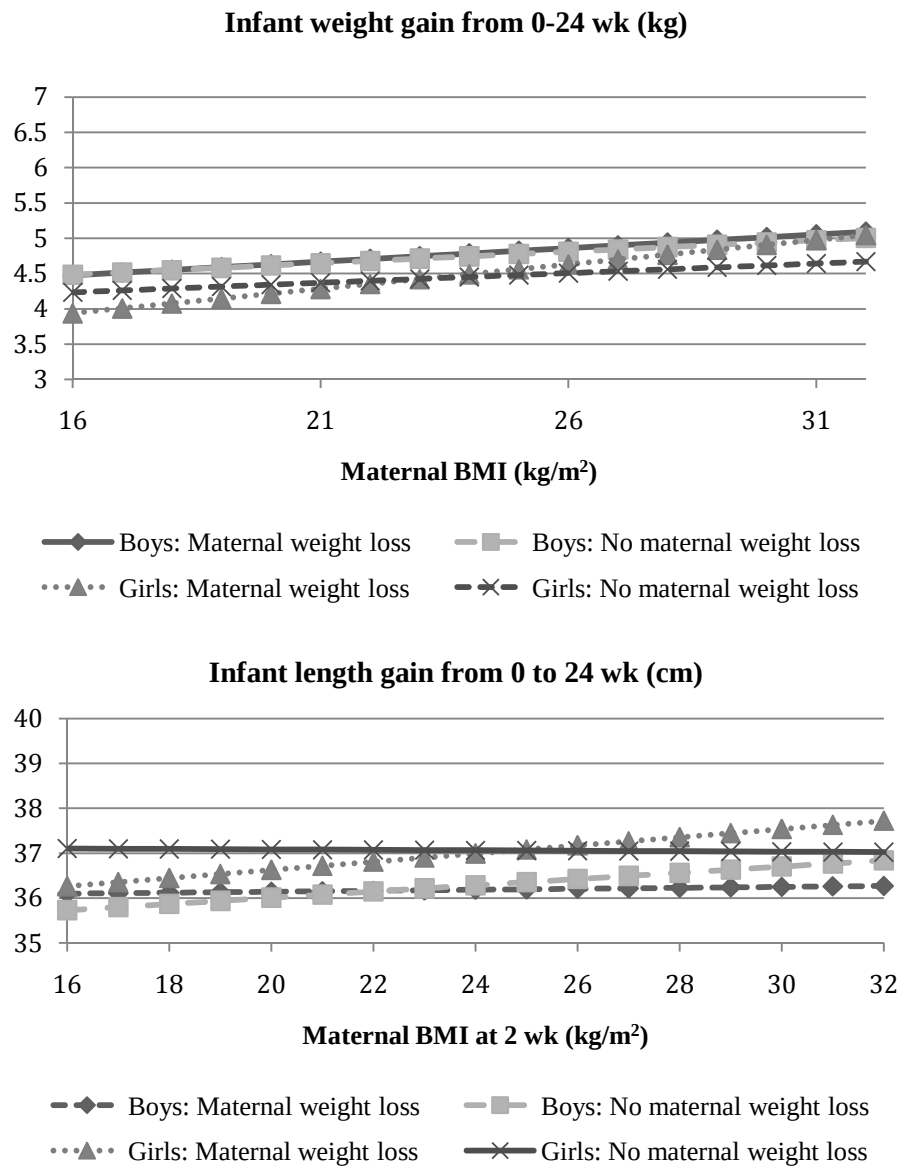

Figure 3.1 Predicted infant weight gain and length from 0 to 24 weeks for varying maternal BMI levels

Predicted values of infant weight and length gain from 0 to 24 weeks from a linear regression model relating maternal weight loss at varying maternal BMI levels at 2 weeks postpartum to infant growth outcomes at 24 weeks. Predicted values at each BMI level represent the linear combination of maternal weight loss coefficient, maternal BMI coefficient and the interaction between maternal weight loss and BMI coefficients. Each model also contained the infant's birth measurement for weight ($p=0.01$) in the weight gain model and length ($p<0.001$) in the length gain model. BMI, body mass index.

Chapter 4. Maternal transferrin receptors and ferritin are associated with infant values in exclusively breastfed HIV-exposed infants

Overview

Infant iron status at birth is influenced by maternal iron status during pregnancy; whether maternal iron status is associated with infant iron status during exclusive breastfeeding is controversial. We evaluated how maternal and infant hemoglobin (Hb) and iron status [transferrin receptors (TfR) and ferritin] were related during exclusive breastfeeding in HIV-infected women and their infants. The BAN (Breastfeeding, Antiretrovirals and Nutrition) Study was a randomized controlled trial in Lilongwe, Malawi, where HIV-infected women were assigned with a 2 x 3 factorial design to lipid-based nutrient supplements (LNS), or no LNS, and maternal antiretrovirals (ARV), infant ARV or no ARV. We used longitudinal models to relate maternal Hb (n=1926) to concurrently measured infant Hb. In a subsample, we regressed change in infant iron status (Hb, log ferritin, log TfR) between 2 (n=355) or 6 wk (n=167), and 24 wk (n=532) on corresponding change in maternal indicator, adjusting for initial values. A one-unit higher maternal Hb at 6, 12, and18 wk was associated with 0.04 g/dL (p=0.02), 0.07 g/dL (p=0.001), and 0.05 g/dL (p=0.008) respective higher value in infant Hb. In the subsample, an increase in maternal TfR and Hb was associated with a respective increase in infant values (TfR β: 0.18 mg/L, p<0.001; Hb: β: 0.13 g/dL, p=0.01). Initial infant values were strong predictors of later infant Hb and iron status (all p-values <0.001). Given the observed maternal influence and the

influence of initial infant values, optimizing maternal iron status is important to protect infant iron status.

Introduction

Iron deficiency is the most common nutrient deficiency in low-income countries and is associated with increased perinatal and maternal mortality, and impaired infant growth, neurodevelopment, and immune function. Given the initial iron endowment at birth, to date the predominant opinion has been that infant iron stores are sufficient during exclusive breastfeeding from birth to 6 months.[63] However, infants in resource poor settings are prone to early depletion of iron stores, especially if maternal iron status was poor before or during pregnancy,[33] and among infants with shorter gestational age,[33] low birthweight,[64,65] male gender,[64] and rapid growth.[33] The identification of postnatal factors that influence infant's risk of early iron store depletion and subsequent deficiency is urgently needed.

Whether maternal iron status is associated with infant iron status during breastfeeding—independent of infant iron stores at birth—is unclear. Studies evaluating the impact of maternal iron status during lactation on breast milk iron content have been inconsistent.[66-70] Several recent studies have shown that maternal Hb and serum iron levels are related to breastmilk iron levels, especially in anemic women or women with impaired iron status markers;[69,70,70-72] however, studies that have investigated whether breastmilk iron levels subsequently influence infant iron status are equivocal.[72]

Understanding the relationship between mother and infant iron status is especially important in the context of HIV. HIV-infected women are at high risk of anemia during pregnancy and of depleted iron stores postpartum.[36,37] In resources poor settings, six months of exclusive breastfeeding by HIV-infected women coupled with antiretroviral prophylaxis to

the mother or infant is recommended to promote child survival and prevent mother-to-child transmission of HIV if replacement feedings are not acceptable, feasible, affordable, sustainable and safe.[45] Together, these factors—impaired maternal iron status, antiretroviral prophylaxis,[73]and exclusive breastfeeding—may result in early depletion of infant iron stores and possibly a greater dependency on breastmilk iron as lactation proceeds.

Our objective was to examine the relationship between maternal and infant hemoglobin (Hb) and iron status (ferritin and TfR), and furthermore because this was part of a randomized controlled trial, to determine the effects of the study interventions on maternal and infant Hb in participants of the Breastfeeding, Antiretrovirals and Nutrition Study (BAN) and a subset of BAN participants selected for additional biomarker assays, the Malawi Mothers and Infants (MaMi) subsample. BAN mother-infant pairs were randomized to a 2-arm nutritional intervention with maternal lipid-based nutrient supplementation (LNS) meeting the nutritional needs of lactation and providing 15 mg iron per day and to a 3-arm antiretroviral (ARV) intervention (maternal, infant or no ARV regimen), and were closely followed during the period of exclusive breastfeeding from 0 to 24 weeks postpartum.

Subjects and Methods

Study population

The data for the current study are from the BAN study, whose design[51] and primary intervention findings have been reported elsewhere.[1,32,49] BAN was a randomized controlled trial of 2,369 mother-infant pairs conducted in Lilongwe, Malawi from April 2004 to February 2010. HIV 1-positive pregnant women (n=3572) were recruited from four antenatal clinics and screened for initial eligibility criteria including: age ≥ 14 years of age, CD4 count ≥ 250 cells/μL (prior to 07/24/2006 CD4 cut off was ≥200 cells/μL in accordance with

Malawi Ministry of Health guidelines for HIV treatment), Hb ≥ 7 g/dL, no prior antiretroviral medication use, and no major pregnancy complications.[1] From the screening visit to 1 week (wk) postpartum, all eligible mothers received daily iron-folic acid supplementation containing 200 mg ferrous sulfate and 5 mg folic acid according to the Malawi Ministry of Health care guidelines. At delivery, eligible women (n=2791) received maternal and infant single dose nevirapine peripartum as well as twice-daily zidovudine and lamivudine for 7 days postpartum. Within 36 hours of delivery, mother-infant pairs had to present at the study site and meet secondary eligibility criteria for randomization including: infant birthweight ≥ 2 kg, no severe congenital malformations, and no other conditions incompatible with survival or that would preclude the use of the study drugs. Of the 2382 mother-infant pairs who met secondary eligibility criteria, thirteen women declined further participation.

Mother-infant pairs (n=2369) were randomized using a permuted-block method to one of six treatment arms according to a two-arm nutritional and three-arm antiretroviral factorial design: maternal LNS/maternal ARV (mLNS-mARV), maternal LNS/infantARV (mLNS-iARV), maternal LNS (mLNS), maternal ARV (mARV), infant ARV (iARV), or control (C).[49] Half of the mothers received a daily maternal LNS dose of 140 g providing estimated added energy and protein requirements of lactation and the recommended daily allowance of micronutrients including 15 mg of iron but excluding vitamin A (Supplemental Table 4.1).[49] The LNS was intended to offset the energy and protein demands of lactation, and was instructed to be eaten in addition to regular foods. The LNS was produced by Nutriset (Malaunay, France) and contained: peanut butter, vegetable oil, dry skimmed milk powder, dry whey, sugar and micronutrients. Mothers in the maternal ARV arms (mLNS-mARV & mARV) received a three-drug highly-active-regimen, while infants in the infant

ARV arms (mLNS-iARV & iARV) received daily oral nevirapine. On March 26, 2008, the data safety monitoring board halted enrollment in the no drug study arms (C & mLNS) because there was evidence that HIV transmission through breastmilk was higher in these groups.[74] Mothers enrolled in these arms were allowed to change to the maternal or infant drug regimen for the remainder of exclusive breastfeeding.[74] Further details of the ARV regimens are described elsewhere.[49,74] Infants diagnosed by HIV-1 with polymerase chain reaction (PCR) within two wk of delivery (n=119) were disenrolled from the study, and mother-infant pairs were referred for care.[1]

In accordance with the World Health Organization prevention of maternal to child transmission of HIV (PMTCT) guidelines in development when the BAN study was designed,[51,53] where exclusive breastfeeding for six months was recommended until acceptable, feasible, affordable, sustainable and safe (AFASS) conditions for replacement feeding are met, intensive counseling regarding exclusive breastfeeding was provided to BAN mothers. Mothers were instructed to exclusively breastfeed their infants for 24 wk and to wean between 24 and 28 wk.[43] All participants were given 2 kg of maize per wk for family consumption to mitigate the effects of seasonal food shortages and to prevent sharing of maternal LNS. The World Food Program (WFP) provided food aid to all HIV-infected women in Lilongwe during a drought between February and August 2005. The aid consisted of a monthly ration of corn/soy flour and 1 liter of vitamin-A-fortified corn oil. An estimated 260 mothers received the ration in lieu of the BAN maize package.[32] Based on WHO recommendations, the BAN study initiated cotrimoxazole preventive therapy for infants 6-36 wk of age in June 2006.[52]

Informed consent was obtained from all participating mothers. The Malawi National Health Science Research Committee and the institutional review boards at University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention approved the study.

Anthropometrics, study procedures and laboratory analyses

Participant visits were conducted at the BAN study clinic at Bwaila Hospital in Lilongwe. Mother-infant pairs were followed at birth and 1, 2, 4, 6, 8, 12, 18, 21, and 24 weeks postpartum, which allows us to closely follow mothers and infants during when breast milk is the sole nutrition source for the infant. At birth and 2, 6, 12, 18, and 24 weeks maternal and infant Hb (g/dL) was measured using Beckman Coulter AcT Differential Analyzer or AcT 5-part Differential Analyzer (Beckman Coulter, Fullerton, CA). Infant HIV status was tested with Amplicor 1.5 DNA PCR at 0, 2, 12 and 28 wk. Positive postnatal HIV tests were confirmed with a specimen obtained at the following visit. Dried blood spots were tested using Gen-Probe Aptima HIV-1 RNA Qualitative assay to further refine the timing of infection in infants. At all visits, maternal and infant weight was measured using regularly calibrated Tanita digital electronic scales to the nearest 0.1 kg unit.Maternal height was measured with a wall-mounted stadiometer. Infant recumbent length was measured using a wooden length board made to UNICEF specifications.[54] Nurses and nutrition assistants were trained in anthropometry and their methods were standardized.[54] Maternal report of parity, marital status, and years of maternal education was obtained at the screening visit. Maternal report of infant's exclusive breastfeeding status was obtained at 4, 8, 12, 18, 21 and 24 wk postpartum.

Plasma was separated from red blood cells, aliquoted to 1 mL plastic storage tubes and stored at -70°C. To identify participants for inflammation and infection biomarker assays (n=537), samples were drawn from the LNS and no LNS groups where all multiple births and infants who became HIV positive at any time point were removed, and mother-infant pairs with anthropometry, and dietary data measurements were prioritized. Ferritin, transferrin receptors (TfR), and markers of inflammation [C-reactive protein (CRP) and α-1-acid glycoprotein (AGP)] concentrations were measured at 2 or 6 and 24 wk postpartum using Cobas Integra 400 (Roche Diagnostics, Indianapolis, IN) in the subsample mother-infant pairs. Initial biomarker measurements were obtained at 2 weeks in infants with sufficient plasma at that time; otherwise assays were conducted with plasma obtained at 6 wk.

Statistical analysis

This paper focuses on two groups of mother-infant pairs in BAN with Hb and iron measurements: mother-infant pairs with longitudinal Hb data (*Longitudinal Hb sample*), and a subset of these mother-infant pairs selected for additional biomarker analyses (*MaMi subsample)*, who have additional measurements of iron status (TfR and ferritin), CRP and AGP. Mother-infant pairs were excluded from analyses if the infant was a multiple (n=49), HIV-infected (n=58). Infants who were weaned early (n=277) were excluded from the longitudinal anlayiss up to the point of cessation of cessation of exclusive breastfeeding. STATA 12.0 (College Station, TX) was used for all statistical analyses.

Longitudinal Hb sample

1926 mother-infant pairs had necessary data for the longitudinal Hb analyses: infant Hb at birth, concurrent maternal and infant Hb at subsequent visits, and birthweight.

Characteristics of the included mother-infant pairs in the *longitudinal Hb sample* (n=1926) were compared to characteristics of randomized mothers excluded from the analysis (n=443) to assess for similarity using t-tests for continuous, normally distributed variables, and nonparametric tests for skewed continuous variables. Compared to excluded randomized mothers, mothers in the *longitudinal Hb sample* were older (26.2 vs. 25.1 years, $p<0.001$), had lower prevalence of low CD4 (29% vs 35%, $p<0.01$), higher pregnancy Hb (10.8 vs. 10.6 g/dL, $p<0.001$) had less anemia during pregnancy (52% vs. 60%, $p=0.002$), and fewer women were primiparous (12 vs. 16%, $p=0.01$). Infants in the *longitudinal Hb sample* had heavier birthweight (3.02 vs. 2.96 kg, $p=0.001$), higher birth length (48.2 vs. 47.9 cm, $p<0.001$), lower prevalence of low birth weight (6% vs. 12%, $p<0.001$), and were more likely to be in the ARV arm (38% vs. 29%, $p<0.001$).

To understand the influence of maternal anemia on infant iron status postpartum, we used linear regression to evaluate whether maternal anemia during pregnancy was associated with infant Hb at birth, adjusting for infant birthweight. We also evaluated whether maternal anemia during pregnancy (Hb <11 g/dL)[75] was associated with infant Hb from 2 to 24 weeks using a longitudinal random effects model with a first order autoregressive disturbance term, adjusting for infant birthweight, birth Hb and rate of weight gain and age. Infant age was modeled with a spline with a knot at 9 weeks of age.

For the primary analysis in the *longitudinal Hb sample*, longitudinal random effects models were utilized to evaluate how maternal Hb related to concurrently measured infant Hb from 2 to 24 wk, modeled with first order autoregressive disturbance terms, adjusting for infant Hb at birth, infant gender, birthweight, and rate of weight gain since preceding visit. To capture the non-linear trend of Hb over time, infant age was modeled with a spline

containing one knot at 9 wks of age. Interactions of age with study interventions, rate of weight gain and infant Hb at birth were evaluated. The BAN study interventions were tested as potential confounders; however, none of the interventions had significant effects compared to mother-infant pairs in the control group (all p-values >0.20) and were not included in the final model.

In secondary analyses in the *longitudinal Hb sample,* we separately evaluated for intervention effects on maternal Hb and infant Hb. In the mothers, a longitudinal random effects model with a first order autoregressive disturbance terms was used to evaluate the effect of the maternal study interventions (mLNS-mARV, mARV, mLNS, control) on maternal Hb from 6 to 24 wk postpartum, adjusting for maternal Hb at 2 wk and time in weeks. Potential interactions with the study interventions and time were evaluated. In the infants, similar longitudinal random effects models were used to evaluate the effect of the six study interventions on infant Hb from 2 to 24 wk. Infant age was modeled a spline containing one knot at 9 wks of age. Potential interactions of study interventions, infant Hb at birth and rate of infant weight gain were also evaluated.

MaMi Subsample

Plots and Shapiro-Wilk tests indicated that plasma ferritin, TfR, CRP, and AGP had a non-Gaussian distribution so these variables were log transformed. Characteristics were compared between mother-infant pairs included in the MaMi subsample (n=537) and excluded mother infant pairs (n=1832) to assess for similarity using t-tests for continuous, normally distributed variables, and nonparametric tests for skewed continuous variables. Compared to the excluded mother-infant pairs, mothers in the *MaMi subsample* were older (26.6 vs. 25.9 years, p=0.01), had lower BMI at delivery (23.3 vs. 23.7 kg/m^2, p=0.02), and

were less likely to be married (90.5% vs. 93.3%, p=0.01) and infants had marginally higher birthweight (3.04 vs. 3.01 kg, p=0.06) and a lower prevalence of low birthweight (4.6% vs. 8% p=0.01), and a larger proportion received the ARV intervention (41% vs. 35%, p=0.02).

Hb and ferritin are sensitive to infection and inflammation—Hb is lowered and ferritin is greatly increased.[76] Though TfR is thought to be less sensitive to inflammation and infection,[76] we observed associations of CRP and AGP with TfR, and therefore adjusted TfR for infection and inflammation. To remove the effects of inflammation from TfR, Hb and ferritin measurements, we used methods proposed by Thurnham and colleagues.[77,78] We used previously used cutpoints to define elevated CRP (>5 mg/L) and AGP (>1 g/L) and stage of inflammation [healthy (normal CRP & AGP), incubation (elevated CRP), early convalescence (CRP & AGP elevated), late convalescence (elevated AGP)].[78] We then determined correction factors for each inflammation group by dividing the median value of the healthy inflammation group by the median value of the incubation, early convalescence, and late convalescence groups.[78] We then multiplied the measured Hb and iron status values by the group specific correction factor.[78]

In the primary *MaMi subsample* analyses, linear regression was used to evaluate how change in maternal iron status (Hb, ferritin and TfR) related to change in infant iron status (Hb, ferritin, or TfR) from 2 (n=355) or 6 wk (n=167) to 24 wk (n=532), controlling for initial maternal and infant values, study ARV arm (mARV or iARV), infant birthweight, and whether the initial values were obtained at 2 or 6 wks postpartum. This approach allows us to theoretically evaluate whether maternal iron status relates to infant status during lactation independent initial maternal influence. Maternal LNS was not included as a covariate in the models in order to isolate the effects of maternal serum status on infant status. We conducted

a sensitivity analysis to evaluate how adjustment for infection and inflammation influenced our findings in the *MaMi subsample* mother-infant pairs. We used linear regression with unadjusted iron markers to evaluate the relationship between change in maternal iron marker on change in infant iron marker outcome, and observed associations similar to the inflammation adjusted models. In addition to these models, we used logistic regression to evaluate whether depleted maternal iron stores (ferritin <15 ug/L) at 2/6 weeks were associated with increased odds of depleted infant iron stores (ferritin <12 ug/L) at 2/6 weeks, adjusting for infant birthweight, ARV arm, when initial measurement was obtained and infant gender.

In secondary analyses in the *MaMi subsample*, we separately evaluated for intervention effects on maternal and infant iron status and Hb, adjusting for infant birthweight and gender. Interactions between LNS and ARV arms were also evaluated. We also conducted logistic regression models to determine if the study interventions were associated with increased odds of impaired infant iron status at 24 weeks, adjusting for initial iron status measurement, birthweight, timing of measurement and infant gender. Interactions between LNS and ARV intervention arms were also evaluated.

Results

In general, mothers in both analyses were young, parous, married and had normal BMI at delivery, but more than half had anemia (Hb<11 g/dL)[75] during pregnancy (Table 4.1). Slightly more than half of the infants were male, few infants had low birthweight and infant Hb was high at birth.

Longitudinal Hb sample

From delivery to 24 wks postpartum, maternal Hb increased (Figure 4.1) and prevalence of maternal anemia (Hb <11 g/dL)[75] decreased from about 50% to 31%. Mean infant Hb followed the typical pattern of decline from birth to 24 weeks, as fetal Hb is broken down[79] (Figure 4.2). From 12-24 wk, prevalence of low infant Hb (<10.5 g/dL)[79] increased from 43% to 50.3%. We observed that pregnancy anemia (Hb<11 g/dL)[75] was associated with lower infant Hb at birth (β: 0.23 g/dL, p=0.01), adjusting for infant birthweight, but was not associated with infant Hb from 2 to 24 weeks in a longitudinal model (β: -0.08 g/dL, p=0.2).

The longitudinal model relating maternal and infant Hb over time indicates that higher maternal Hb predicts higher infant Hb over time (Supplemental Table 4.2) with significant associations between 6 and 18 wks (Figure 4.3). Female gender and higher birthweight were associated with higher infant Hb (p<0.001). Higher infant Hb at birth was associated with higher infant Hb over time, while faster weight gain—especially in younger infants—was associated with lower infant Hb over time.

Effects of the study interventions

LNS intervention effects were minimal on maternal Hb, while there were some adverse but not sustained effects of maternal ARV provision over time in both the mARV and mLNS-mARV groups (Supplemental Table 4.3). Among mothers who received no ARVs, there were no differences in maternal Hb over time between women who received or did not receive LNS (all p-values >0.75). For mothers who received both LNS and ARVs (mLNS-mARV), predicted values from the longitudinal models indicate that maternal Hb was significantly lower at 6 and 12 wks postpartum (6 wk β: -0.32, p<0.001, 12 wk β: -0.20,

p<0.001); however, at 18 and 24 wks the negative effects were not sustained (all p-values >0.25). Similar effects were observed in mothers who received only ARVs (mARV), where maternal Hb was significantly lower at 6 and 12 wk postpartum (6 wk β: -0.22, p=0.001, 12 wk β: -0.15, p=0.003), but again at 18 and 24 wks the negative effects were not sustained (all p-values >0.1). The longitudinal model of infant Hb shows no effects of the intervention arms on infant Hb (Supplemental Table 4.4).

MaMi subsample

In the *MaMi subsample,* inflammation adjusted maternal Hb remained relatively stable from initial measurement to 24 wk, while maternal TfR declined (Table 4.2). Initially, prevalence of depleted maternal iron stores (adjusted ferritin <15 ug/L) was high, while fewer women had tissue iron depletion, denoted by elevated adjusted TfR (TfR >8.3 mg/L). By 24 weeks, about a third of women had depleted iron stores, but again fewer women had tissue iron depletion. Inflammation adjusted infant Hb declined from initial measurement to 24 wk. Infant ferritin values declined markedly, while TfR increased from initial measurement to 24 wk. Few infants had abnormal initial adjusted measurements, but by 24 wk many infants began to show signs of poor iron status. From initial measurement to 24 weeks, prevalence of elevated CRP (>5 mg/L) and AGP (>1 g/L) declined in mothers and increased in infants.

Maternal TfR and Hb, but not ferritin, influenced infant values (Table 4.3). An increase in maternal TfR was associated with an increase in infant TfR, suggesting that worsening maternal iron status was related to worsening infant iron status. Similarly, an increase in maternal Hb was associated with an increase in infant Hb at 24 wk. In all models, initial infant values were the strongest predictor of change from the initial measurement.

Higher birthweight and female gender were strongly associated with lower TfR, but higher Hb and ferritin. In these models relating change in maternal value to change in infant value, maternal and infant ARV regimens had no effects on infant TfR, Hb or ferritin. In models relating maternal iron store depletion to odds of depleted infant iron stores, we observed that depleted maternal iron stores (ferritin <15 ug/L) at 2/6 weeks were not associated with depleted infant iron stores at both 2/6 and 24 weeks (ferritin <12 ug/L) (2/6 wk: OR: 2.23, p=0.32; 24 wk: 0.98, p=0.94).

Effects of the study interventions

mLNS was not related to maternal Hb, TfR, or Ferritin values at 2, 6, or 24 wk (all p-values >0.1) (Table 4.4). mARV prophylaxis was associated with lower maternal Hb at 6 wk (p<0.001), but was unrelated to maternal ferritin (all p-values >0.05). mARV prophylaxis was associated with higher maternal TfR (suggestive of worsening iron status) at 6 and 24 wks; however, the adverse ARV effect was reduced if the mother received LNS (Figure 4.4).

mLNS had no effects on infant Hb at any time (all p-values >0.1). mARV prophylaxis was associated with lower infant Hb at 2 wk, but not at later times. Similarly, iARV was associated with lower infant Hb only at 2 wk. We also evaluated whether the interventions were associated with odds of infant anemia at 24 weeks (Hb <10.5 g/dL), and observed a significant interaction between mLNS and iARV (Table 4.5); however, based on linear combinations of the mLNS, iARV and mLNS*iARV coefficients the mLNS coupled with iARV was not associated with increased odds of anemia (OR: 1.20, p=0.58).

mLNS had no effects on infant ferritin initially and was associated with lower infant ferritin at 24 weeks but not increased odds of infant iron store depletion (ferritin <12 ug/L) at 24 weeks. mARV or iARV prophylaxis had no effects on infant ferritin. mLNS was

associated with higher infant TfR at 2 wk, but not at 6 or 24 weeks. At 2 wk, a significant interaction was observed between mLNS and mARV (p=0.07) as well as between mLNS and iARV (p=0.01) on infant TfR outcomes. Based on the linear combinations of mLNS, iARV and mLNX*iARV coefficients, the net effect of provision of mLNS along with iARV was not higher infant TfR (β: 0.17, p=0.15). On the other hand, based on the linear combinations of the mLNS, mARV and mLNS*mARV coefficients, the effect of mLNS provided with mARV was marginally higher infant TfR (β: 0.26, p=0.04). We tested whether the interventions were associated with increased odds of abnormal TfR (TfR>8.3), and observed no association.

Discussion

In our study of HIV-infected Malawian mothers and their infants during exclusive breastfeeding, maternal Hb and TfR predicted infant values. More than half of mothers had anemia during pregnancy, which was associated with lower infant Hb at birth. This is important because the infant's iron endowment at birth was related to subsequent risk of iron depletion postpartum. Initial maternal iron measurements indicate that despite routine supplementation during pregnancy, maternal iron stores were depleted in many women. Initial infant iron measurements suggest that the mechanisms to protect infant iron status were effective in the immediate postpartum period,[71] since mean infant Hb at birth was within normal levels and few infants had depleted iron stores or high TfR initially. At 24 wk, however, infant iron status was poor, suggesting that initial infant stores at birth were insufficient for the period of exclusive breastfeeding and possibly that breastmilk iron levels may have been suboptimal. Interestingly, we observed no association between low maternal

ferritin at 2/6 weeks and low infant ferritin at 2/6 or 24 weeks; suggesting that breastmilk iron levels may have been protected in some mothers with impaired iron status.

In the *longitudinal* Hb mother-infant Hb models, higher maternal Hb predicts higher infant Hb between 6 and 18 wk. Similarly in the *MaMi subsample* where we corrected Hb measurements for infection and inflammation, larger increases in maternal Hb were associated with larger changes in infant Hb; providing further evidence that maternal Hb predicts infant Hb during exclusive breastfeeding.[79] Conversely, larger increases maternal TfR predicted larger changes in infant TfR during breastfeeding, suggesting that worsening maternal iron status contributes to worsening infant status. Maternal iron stores must be depleted for tissue iron depletion to manifest as increased TfR levels, thus our observed association between maternal and infant TfR suggests that mothers with severely impaired iron status may have impaired breastmilk iron levels. We did not observe an association between maternal and infant ferritin, which has been previously observed.[80] Infant iron stores at birth are regulated to be high in order to ensure adequate iron stores during breastfeeding; in our analysis this is apparent by the high infant ferritin values at 2 or 6 weeks. Given the underlying mechanisms to protect and maximize iron transfer to the fetus even with the presence of maternal anemia,[81] and the many factors influence infant iron endowment at birth including gestational age,[33] and timing of cord clamping[82], the lack of an association between maternal and infant ferritin is not surprising and is further supported by the absence of an association between the initial maternal ferritin measurement and infant ferritin at 24 wk.

In the *longitudinal Hb sample*, the BAN study interventions—especially the ARV regimen—had some effect on maternal Hb. Though maternal LNS had 15 mg of iron, this may not have been enough to replete the iron stores of mothers with impaired iron status

postpartum. As such, our lack of LNS main effects was anticipated. Though we observed that ARVs provided with or without LNS had negative effects on maternal HB at 6 and 12 weeks, these effects were not sustained at later times. In the *MaMi subsample*, in contrast to our longitudinal model findings, we observed no interactions between mLNS and mARV on maternal Hb; but observed negative ARV effects on maternal Hb at 2 wk postpartum. Provision of maternal LNS mitigated some of the adverse effects of ARVs on maternal TfR values, indicating that LNS provision was protective for maternal iron status.

Even though we observed some effects of the BAN study interventions on maternal Hb, the study interventions had no influence on infant Hb values in the *longitudinal Hb sample*. This is consistent with a previous study in Turkish women, where maternal iron supplementation had little effect on milk iron content, the milk-maternal serum iron ratio or infant iron status;[72,72] but women with anemia (Hb <11 g/dL) (who would have likely received the most benefit from the supplement) were excluded from the study.

In the *MaMi subsample,* we observed mixed effects of the study interventions on maternal and infant Hb and iron status. mLNS was not associated with maternal Hb, ferritin or TfR, while mARV provided with or without mLNS was associated with impaired maternal TfR. The study interventions were not associated with infant Hb at any time or with increased odds of anemia at 24 weeks. Though mLNS was associated with lower infant ferritin at 24 wk, it was not associated with increased odds of depleted infant iron stores (ferritin <12 ug/L) at 24 wk. We observed that provision of LNS coupled with maternal ARVs was associated with marginally higher infant TfR at 2 wk, but not at other times. However, the study interventions were not associated with increased odds of elevated TfR.

This research has some limitations. First, we lacked measures of the acute phase response in the *longitudinal Hb sample* and were not able to adjust for inflammation and infection in this group. Because Hb is lowered in the presence of inflammation and infection, presence of illness or subclinical inflammation may have distorted our *longitudinal Hb sample* findings. In our sensitivity analysis to evaluate how adjustment for infection and inflammation influenced our findings in the *MaMi subsample* mother-infant pairs, we observed associations similar to the inflammation adjusted models. This suggests that our *longitudinal Hb model* findings are likely not to be markedly affected by inflammation and infection. Second, we do not know whether mothers received and took iron supplements during pregnancy; however, by controlling for the mother's initial measurement this may not have biased our findings.

In the *MaMi subsample* we have initial measurements of iron status and Hb at two time points due to insufficient plasma availability at 2 weeks postpartum. During this time period, infant iron status, particularly Hb, changes dramatically. We utilized a dummy variable for time of measurement in our primary models in order to retain our full MaMi subsample for the change analyses, which was influential in the TfR and Hb models. To evaluate intervention effects, we used linear regression at each time point and may not have had sufficient power to detect LNS effects with initial measurements, as the sample at 2 weeks may have been too small to detect associations, as the mothers in the mLNS group only had 2 weeks of LNS consumption.

In the *longitudinal Hb sample, w*e excluded about 19% of randomized mother-infant pairs from our longitudinal analysis if they did not have measurements required for inclusion in the analysis (birthweight, birth Hb, and concurrently measured mother-infant Hb) (n=

217), or were multiple births (n=49), HIV-infected in first 2 wk (n=119) or HIV-infected from 2 to 24 wk (n=58). Infants weaned early were censored when maternal reports indicated that the infant was no longer exclusively breastfed (n=277). Compared to infants included in the *longitudinal Hb sample*, worse off infants and mothers were excluded or lost-to-follow up. Similarly, mother-infant pairs in the *MaMi subsample* were healthier than randomized infants. As such, we may have underestimated the observed relationships between maternal and infant Hb and iron status.

This study also has many strengths. This is the first study to characterize the relationship between maternal and infant iron status using multiple iron measures during exclusive breastfeeding. In these analyses, we controlled for the infant's initial measurement; therefore we are theoretically observing the effects of breastmilk iron, as breastmilk was the sole source of nutrition for BAN infants during this time. Applying a dyadic modeling framework is an innovative approach to examine these mother-infant iron status relationships. Supporting this approach are our detailed exclusive breastfeeding data, which allowed us to exclude weaned and mixed fed infants from our analyses, as the mother was no longer the sole source of nutrition for her growing infant and infants who potentially received other sources of iron in complementary foods were also excluded. Moreover, our analysis focused on the early postpartum period, a period where infant iron status is not well characterized, and adds to our understanding of iron status dynamics during this time. Finally, understanding these relationships is especially important in the context of HIV to guide future interventions and programs to promote maternal and infant health and to optimize maternal and infant iron status.

The LNS and ARV interventions had few sustained effects on maternal and infant Hb and iron status over time in the *longitudinal Hb sample*. In the *MaMi subsample,* we observed that the mLNS and the mLNS-mARV interventions were associated with impaired TfR and ferritin values, respectively; however, they were not associated with increased odds of abnormal values. The current WHO recommendations for infant feeding in the context of HIV is forsix months of exclusive breastfeeding followed by introduction of appropriate complementary foods with continued breastfeeding for 12 months, along with provision of ARV prophylaxis to the mother or infant up to 1 wk after cessation of breastfeeding. Given these recommendations along with the high nutritional demands of lactation and HIV, providing LNS with only 15 mg of iron along with ARVs to HIV-infected lactating women may have little clinical significance for maternal and infant iron status, but LNS still may be valuable to support breastfeeding in HIV-infected mother in resource poor settings.[49] LNS may be particularly advantageous in places with corn-based staple foods, such as nsima in Malawi, since phytates present in corn may inhibit iron absorption and increase likelihood of poor iron status.

Given the observed association between maternal and infant Hb and TfR, and the strong sustained effects of infant's birth Hb in the *longitudinal Hb sample* and initial iron status measurements in the *MaMi subsample* on later status, optimizing maternal iron status is important for reducing risk of iron depletion in infants. Further research to understand how iron status markers relate to breastmilk iron levels appear warranted. Moreover, further research to understand how ARVs affect maternal and infant iron status from 6 to 12 months is needed.

Table 4.1 Characteristics of mother-infant dyads in both analysis samples

	Longitudinal Hb Sample n=1926	MaMi Subsample n=519[1]
MOTHERS		
Age (y)[2]	26.2 ± 5.0	26.6 ± 5.2
Height (cm)[2]	156.88 ± 5.52	156.57 ± 5.40
BMI at 2 wk postpartum (kg/m^2)[2]	23.59 ± 3.03	22.85 ± 2.98
CD4 (cells/µL)[2,3]	480.97 ± 197.92	482.63 ± 221.68
CD4 < 350 cells/µL (%)	28.6	30.1
Hb (g/dL) [2,3]	10.8 ± 1.22	10.80 ± 1.18
Anemia (%)[3]	52.0	53.8
Primaparous (%)	11.6	10.3
Married (%)	92.3	90.2
Education (%)	35.0	36.8
INFANTS		
Female (%)	49.3	47.6
Birthweight (kg)[2]	3.03 ± 0.40	3.05 ± 0.39
Low birthweight (%)	6.2	4.6
Birth length (cm)[2]	48.22 ± 1.94	48.23 ± 1.96
Hb at birth (g/dL)[2]	17.44 ± 2.02	17.34 ± 2.09
STUDY INTERVENTION (%)[4]		
mLNS-mARV	17.7	16.8
mARV	17.6	15.8
mLNS-iARV	19.2	20.0
iARV	18.5	20.4
mLNS	13.2	14.5
C	13.9	12.5

[1]Though 537 mother-infant pairs were included in the MaMi subsample, 18 of these pairs were missing mother or infant inflammatory marker measurements and were not included in this analysis

[2]Mean ± SD

[3]Measured during pregnancy, Anemia defined as Hb <11 g/dL[75]

[4]Study intervention: maternal LNS/maternal ARV (mLNS-mARV), maternal LNS/infantARV (mLNS-iARV), maternal LNS (mLNS), maternal ARV (mARV), infant ARV (iARV), or control (C)

Table 4.2 Infection, Hb and inflammation adjusted and unadjusted markers of iron status of MaMi subsample mother-infant dyads[1]

	Initial measurement				24 week	
	2 week		6 week			
	Unadjusted	Adjusted[2]	Unadjusted	Adjusted[2]	Unadjusted	Adjusted[2]
MOTHER						
Hb(g/dL)[3]	11.9 ± 1.6 (365)	12.6 ± 1.6 (360)	12.0 ± 1.4 (170)	12.2 ± 1.3 (170)	12.4 ± 1.2 (535)	12.6 ± 1.2 (534)
Adverse event (%)[4]	11.2	6.4	7.7	6.47	2.99	1.5
Low (%)[4]	47.4	35.28	42.35	38.82	34.77	29.59
Ferritin (ng/mL)[3]	46.3 ± 55.2 (367)	29.98 ± 37.26 (362)	42.83 ± 65.27 (170)	43.09 ± 63.69 (170)	34.65 ± 47.82 (537)	32.38 ± 43.08 (536)
Deficient(%)[4]	23.98	39.23	33.53	32.35	31.28	33.4
TfR (mg/L)[3]	5.68 ± 2.80 (367)	5.69 ± 2.82 (362)	5.98 ± 3.70 (170)	5.67 ± 3.50 (170)	5.09 ± 2.42 (536)	4.86 ± 2.27 (535)
Deficient(%)[4]	13.08	12.71	18.24	15.88	8.77	6.92
Elevated inflammatory markers						
CRP >5 mg/L (%)	42.9	-	22.4	-	16.4	-
AGP >1 g/L (%)	76.8	-	47.7	-	33.5	-
INFANT						
Hb (g/dL)[3]	14.25 ±1.79 (357)	14.30 ± 1.78 (351)	11.05 ± 1.30 (165)	11.14 ± 1.28 (163)	10.39 ± 1.11 (530)	10.47 ± 1.10 (526)
Adverse event(%)[5]	26.89	26.21	10.91	9.82	56.98	54.37
Low(%)[5]	-	-	-	-	71.7	69.39
Ferritin (ng/mL)[3]	479.5 ± 354.3 (359)	462.7 ± 338.7 (359)	322.11 ± 238.53 (168)	314.67 ± 228.92 (167)	40.02 ± 94.34 (537)	42.34 ± 102.80 (533)
Deficient(%)[5]	1.95	1.39	6.55	2.99	32.96	30.96
TfR(mg/L)[3]	3.56 ± 1.94 (360)	3.53 ± 1.90 (360)	3.55 ± 2.13 (165)	3.35 ± 1.91 (165)	6.88 ± 3.47 (532)	6.64 ± 3.33 (531)
Deficient(%)[5]	1.67	1.67	3.64	3.03	24.44	21.85
Elevated inflammatory markers						
CRP >5 mg/L (%)	8.9	-	16.2	-	28.5	-
AGP >1 g/L (%)	8.6	-	16.1	-	47.0	-

[1]Hb, serum hemoglobin; TfR, plasma soluble transferrin receptor; CRP, plasma C-reactive protein; AGP, plasma α_1-acid glycoprotein.

[2]Adjusted for inflammation by using group specific correction factors estimated from ratios of medians for the various iron indicators[77]

[3]Values are Mean ± SD (n)

[4]Adverse event for maternal Hb was defined as <10.0 g/dL,[83] low <12 g/dL,[37] abnormal ferritin <15 ng/mL, abnormal TfR>8.3 mg/L

[5]Adverse event for infant Hb is <13 g/dL from 1-21 d, <10.5 g/dL 22-35 d, <9.4g/dL from 36-56 d, <10.9 g/dL ≥56 d.[83] Low infant Hb was defined as <10.5 g/dL.[79] Abnormal infant TfR >8.3 mg/L and ferritin <12 ng/mL.

Table 4.3 Linear regression models showing the effects of change in maternal iron status on change in infant iron status outcomes (log transferrin receptors, hemoglobin, log ferritin) from 2/6 to 24 weeks, adjusted and unadjusted for inflammation[1,2]

	Log TfR (n=513)			Hb (n=496)			Log Ferritin (n=518)		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
Inflammation Adjusted									
Change in maternal value	0.18	[0.06, 0.29]	<0.001	0.13	[0.03, 0.23]	0.01	0.06	[-0.06, 0.17]	0.34
Infant initial value	-0.66	[-0.72, -0.60]	<0.001	-0.89	[-0.94, -0.83]	<0.001	-0.92	[-1.01, -0.83]	<0.001
Maternal initial value	0.23	[0.14, 0.33]	<0.001	0.1	[0.02, 0.18]	0.02	0.03	[-0.08, 0.14]	0.61
Infant birthweight	-0.23	[-0.32, -0.14]	<0.001	0.46	[0.22, 0.70]	<0.001	0.34	[0.13, 0.56]	<0.001
mARV[3]	-0.01	[-0.10, -0.09]	0.85	0.1	[-0.14, 0.34]	0.41	0.14	[-0.08, 0.35]	0.22
iARV[3]	0.03	[-0.05, 0.12]	0.43	-0.11	[-0.35, 0.12]	0.34	-0.1	[-0.30, 0.11]	0.35
Initial measure at 6 wk	0.21	[0.13, 0.28]	<0.001	0.32	[0.05, 0.59]	0.02	0.13	[-0.06, 0.31]	0.18
Female gender	-0.19	[-0.26, -0.12]	<0.001	0.15	[-0.04, 0.34]	0.11	0.45	[0.28, 0.62]	<0.001
Inflammation unadjusted									
Change in maternal value	0.18	[0.06, 0.29]	0.002	0.13	[-.03, 0.23]	0.01	0.06	[-1.01, -0.83]	0.34
Infant initial value	-0.66	[-0.72, -0.60]	<0.001	-0.89	[-0.94, -0.83]	<0.001	-0.92	[-0.08, 0.14]	<0.001
Maternal initial value	0.23	[0.14, 0.33]	<0.001	0.10	[0.02, 0.18]	0.02	0.03	[0.13, 0.56]	0.61
Infant birthweight	-0.23	[-0.32, -0.14]	<0.001	0.46	[0.22, 0.70]	<0.001	0.34	[-0.08, 0.35]	0.00
mARV[3]	-0.01	[-0.10, 0.09]	0.85	0.10	[-0.14, 0.34]	0.41	0.14	[-0.30, 0.11]	0.22
iARV[3]	0.03	[-0.05, 0.12]	0.43	-0.11	[-0.35, 0.12]	0.34	-0.10	[-0.06, 0.31]	0.35
Initial measure at 6 wk	0.21	[0.13, 0.28]	<0.001	0.32	[-0.05, 0.59]	0.02	0.13	[0.28, 0.62]	0.18
Female gender	-0.19	[-0.26,-0.12]	<0.001	0.15	[-0.04, 0.34]	0.11	0.45	[-0.19, 1.68]	<0.001

[1]LNS arm was not included in the model to isolate the effects of maternal iron status independent of study supplement

[2]Hb, serum hemoglobin; TfR, plasma soluble transferrin receptor; CRP, plasma C-reactive protein; AGP, plasma α_1-acid glycoprotein.

[3]mARV(maternal ARV), iARV (infant ARV)

Table 4.4 Associations between the BAN study interventions and maternal and infant Hb, TfR and ferritin outcomes in MaMi subsample[1]

	Initial measurement						24 weeks		
	2 weeks			6 weeks					
	Coef.	95% CI	p-value	Coef.	95% CI	p-value	Coef.	95% CI	p-value
MOTHER									
Hb (g/dL)		n=360			n=170			n=534	
mLNS	-0.03	[-0.37, 0.30]	0.85	-0.03	[-0.43, 0.36]	0.88	-0.01	[-0.20, 0.19]	0.94
mARV	-0.01	[-0.38, 0.35]	0.94	-0.72	[-1.13, -0.32]	0.001	-0.02	[-0.23, 0.19]	0.86
Log Ferritin (ng/mL)		n=362			n=170			n=536	
mLNS	-0.06	[-0.25, 0.13]	0.53	0.09	[-0.20, 0.37]	0.55	0.04	[-0.10, 0.18]	0.53
mARV	0.17	[-0.03, 0.38]	0.10	-0.25	[-0.55, 0.04]	0.09	-0.06	[-0.21, 0.09]	0.45
Log TfR (mg/L)		n=362			n=170			n=535	
mLNS	0.03	[-0.08, 0.14]	0.61	0.15	[-0.04, 0.35]	0.11	0.02	[-0.06, 0.10]	0.64
mARV	-0.11	[-0.25, 0.04]	0.15	0.36	[0.15, 0.57]	0.001	0.25	[0.15, 0.35]	<0.001
mLNS*mARV	0.03	[-0.18, 0.23]	0.81	-0.27	[-0.58, 0.03]	0.08	-0.11	[-0.26, 0.03]	0.13
INFANT									
Hb (g/dL)		n=351			n=163			n=526	
Birthweight	0.23	[-0.26, 0.72]	0.35	0.60	[0.10, 1.10]	0.02	0.58	[0.34, 0.82]	<0.001
Female	0.35	[-0.03, 0.73]	0.07	0.31	[-0.10, 0.72]	0.13	0.17	[-0.02, 0.36]	0.07
mLNS	-0.25	[-0.62, 0.13]	0.20	-0.05	[-0.45, 0.34]	0.79	-0.13	[-0.32, 0.06]	0.18
mARV	-0.47	[-0.94, 0.003]	0.05	-0.07	[-0.63, 0.49]	0.80	0.02	[-0.22, 0.26]	0.89
iARV	-0.73	[-1.17, -0.28]	0.001	-0.36	[-0.91, 0.19]	0.20	-0.21	[-0.44, 0.02]	0.07
Log Ferritin (ng/mL)		n=359			n=167			n=533	
Birthweight	0.19	[-0.05, 0.43]	0.12	0.17	[-0.24, 0.59]	0.41	0.36	[0.15, 0.57]	0.001
Female	0.16	[-0.02, 0.34]	0.08	0.24	[-0.10, 0.57]	0.16	0.47	[0.31, 0.64]	<0.001
mLNS	-0.12	[-0.30, 0.06]	0.20	-0.27	[-0.60, 0.05]	0.10	-0.21	[-0.37, -0.05]	0.01
mARV	0.03	[-0.20, 0.26]	0.79	0.15	[-0.31, 0.61]	0.52	0.17	[-0.04, 0.38]	0.11
iARV	-0.04	[-0.25, 0.17]	0.71	0.26	[-0.20, 0.71]	0.27	-0.08	[-0.28, 0.12]	0.42
Log TfR (mg/L)		n=360			n=165			n=531	
Birthweight	0.02	[-0.15, 0.20]	0.78	-0.14	[-0.32, 0.05]	0.15	-0.26	[-0.36, -0.15]	<0.001
Female	-0.17	[-0.31, -0.03]	0.01	-0.12	[-0.27, 0.03]	0.12	-0.26	[-0.34, -0.18]	<0.001
mLNS	0.35	[0.11, 0.59]	0.004	0.05	[-0.10, 0.19]	0.53	-0.01	[-0.09, 0.07]	0.87
mARV	0.23	[-0.02, 0.48]	0.08	0.04	[-0.17, 0.25]	0.70	0.05	[-0.05, 0.16]	0.33
iARV	0.23	[-0.002, 0.47]	0.05	-0.03	[-0.24, 0.17]	0.76	0.05	[-0.05, 0.15]	0.36
mLNS*mARV	-0.31	[-0.65, 0.03]	0.07		-			-	
mLNS*iARV	-0.42	[-0.74, -0.10]	0.01		-			-	

[1]Linear regression models tested for intervention associations with maternal and infant Hb and iron status outcomes. We tested for significant interactions between mLNS and mARV/iARV, if significant (p<0.1) the interaction term was retained in the model. Hb, hemoglobin; TfR, transferrin receptors; BAN, Breastfeeding Antiretroviral and Nutrition; MaMi, Malawi Mothers and Infants; mLNS, maternal LNS; mARV, maternal ARV; iARV, infant ARV.

Table 4.5 Associations between study interventions and odds of impaired infant iron status at 24 weeks in the MaMi subsample[1]

	High TfR			Low Hb			Low ferritin		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Initial measurement	9.16	[4.77, 17.59]	<0.001	0.79	[0.70, 0.89]	<0.001	0.64	[0.52, 0.79]	<0.001
Birthweight	0.32	[0.17, 0.62]	<0.001	0.30	[0.18, 0.50]	<0.001	0.40	[0.24, 0.69]	<0.001
mARV	1.25	[0.66, 2.36]	0.49	0.97	[0.49, 1.94]	0.94	0.79	[0.47, 1.33]	0.37
iarV	1.50	[0.82, 2.75]	0.19	1.50	[0.77, 2.91]	0.24	1.29	[0.80, 2.08]	0.30
mLNS	0.95	[0.60, 1.52]	0.84	1.78	[0.87, 3.63]	0.11	1.18	[0.80, 1.75]	0.40
mLNS*mARV		-		0.52	[0.20, 1.35]	0.18		-	
mLNS*iARV		-		0.45	[0.18, 1.14]	0.09		-	
Initial measurement at 6 wk	3.50	[2.12, 5.75]	<0.001	0.37	[0.21, 0.64]	<0.001	0.59	[0.38, 0.92]	0.02
Female	0.53	[0.32, 0.86]	0.01	0.67	[0.46, 0.98]	0.04	0.44	[0.29, 0.66]	<0.001

[1]Logistic regression models tested for intervention effects with impaired infant iron status outcomes. We tested for significant interactions between mLNS and mARV/iARV, if significant ($p<0.1$) the interaction term was retained in the model.

[2]Impaired infant iron status:Low Hb <10.5 g/dL[79] & low ferritin <12 ng/mL, high TfR >8.3. Hb, hemoglobin; TfR, transferrin receptors; BAN, Breastfeeding Antiretroviral and Nutrition; MaMi, Malawi Mothers and Infants; mLNS, maternal LNS; mARV, maternal ARV; iARV, infant ARV.

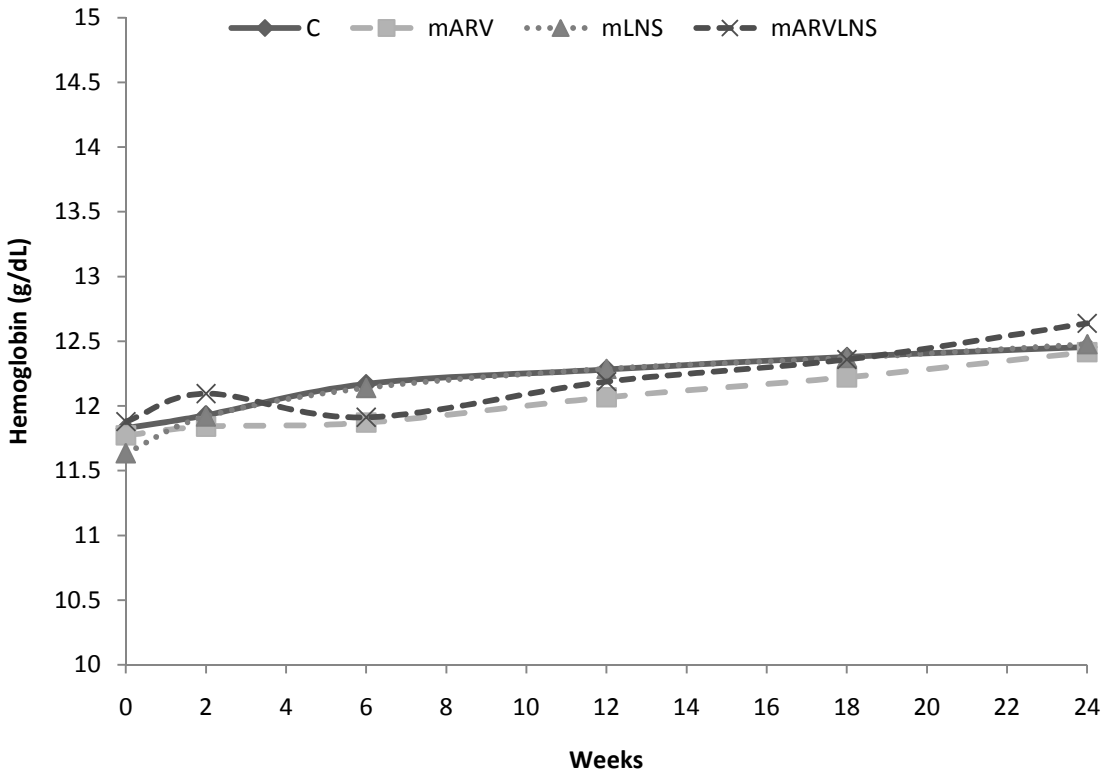

Figure 4.1 Mean maternal hemoglobin (g/dL) values according to BAN study arm from birth to 24 weeks

Data among BAN mothers in the longitudinal Hb sample with at least one concurrent mother and infant Hb measurement (n=623 in the C arm; n=338 in the mARV arm; n=624 in the mLNS arm; n=341 in the maternal ARV-LNS arm). mARVLNS, maternal LNS/maternal ARV; mLNS, maternal LNS; mARV, maternal ARV; C, control; BAN, Breastfeeding, Antiretroviral and Nutrition; LNS, lipid-based nutrient supplement; ARV, antiretroviral drug.

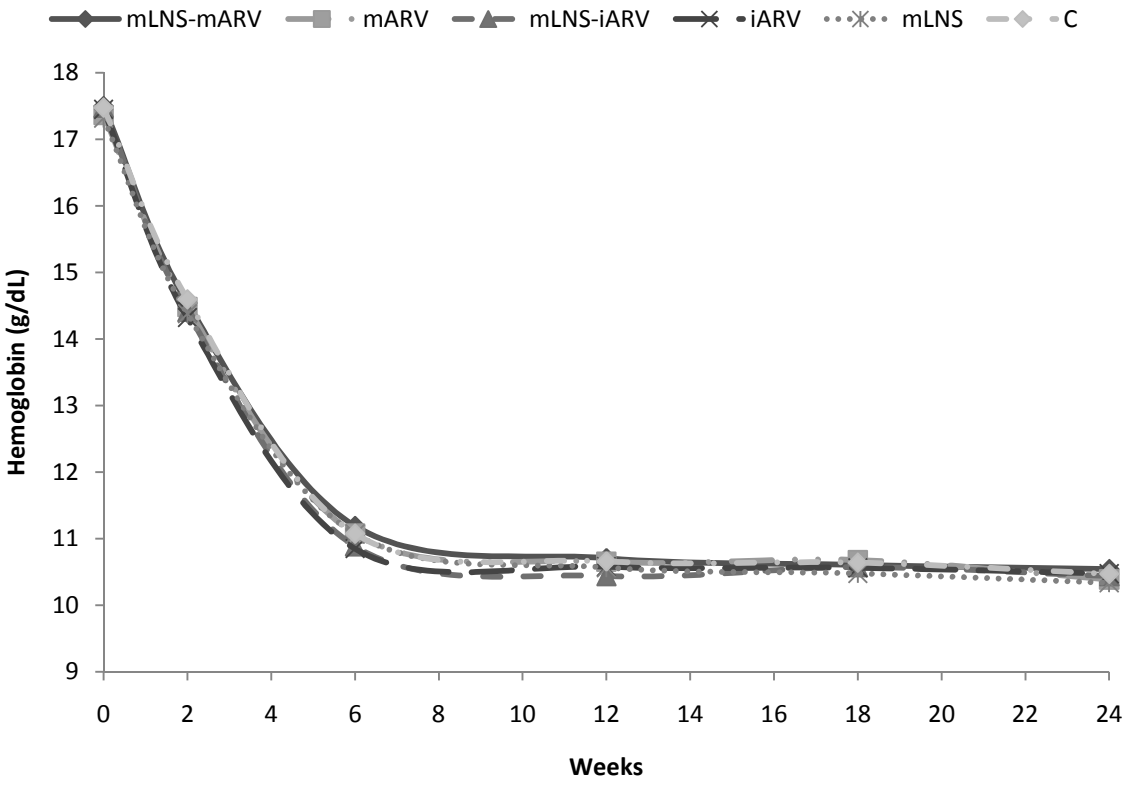

Figure 4.2 Infant hemoglobin (g/dL) values according to BAN study arm from birth to 24 weeks

Data among BAN infants in the longitudinal Hb sample with at least one concurrent mother and infant Hb measurement (n=341 in the mLNS-mARV arm; n=338 in the mARV arm; n=369 in the mLNS-iARV arm; n=356 in the iARV arm; n=255 in the mLNS arm; n=267 in the C arm). mLNS-mARV, maternal LNS/maternal ARV; mARV, maternal ARV; mLNS-iARV, maternal LNS/infant ARV; iARV, infant ARV; mLNS, maternal LNS; C, control. BAN, Breastfeeding, Antiretroviral and Nutrition; LNS, lipid-based nutrient supplement; ARV, antiretroviral drug.

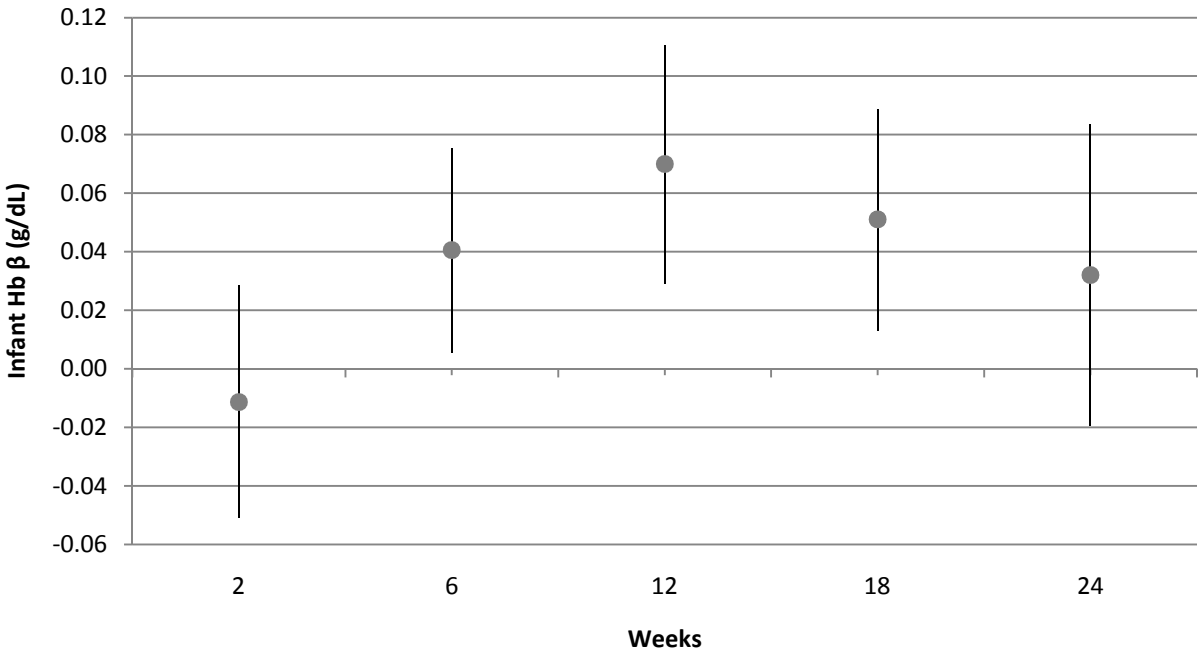

Figure 4.3 Predicted infant Hb coefficients (β ± 95% Confidence Interval) for each age in BAN infants

Predicted Hb values (g/dL) from a longitudinal random effects model model relating maternal Hb to infant Hb containing a spline with a knot at 9 weeks to capture the shape of infant Hb over time. Predicted values represent at each age the linear combination of the maternal Hb coefficient and the maternal Hb interactions with age. The model also contained infant age ($p<0.001$), and infant age after 9 weeks ($p<0.001$), female gender ($p<0.001$), infant birthweight ($p<0.001$), infant Hb at birth ($p<0.001$), and rate of weight gain since previous visit ($p<0.001$). Interaction terms with age and age >9 wk included: infant birth Hb ($p<0.001$) rate of weight gain since previous visit ($p<0.001$). Data from HIV-negative infants and their mothers with at least one concurrent mother infant Hb measurement and birth Hb were included until 24 weeks or cessation of exclusive breastfeeding: n=341 in the mLNS-mARV arm; n=338 in the mARV arm; n=369 in the mLNS-iARV arm; n=356 in the iARV arm; n=255 in the mLNS arm; n=267 in the C arm. BAN, Breastfeeding, Antiretroviral and Nutrition; mLNS-mARV, maternal LNS/maternal ARV; mARV, maternal ARV; mLNS-iARV, maternal LNS/infant ARV; iARV, infant ARV; mLNS, maternal LNS; C, control. Hb, Hemoglobin; BAN, Breastfeeding Antiretoviral and Nutrition; LNS, lipid-based nutrient supplement; ARV, antiretroviral intervention.

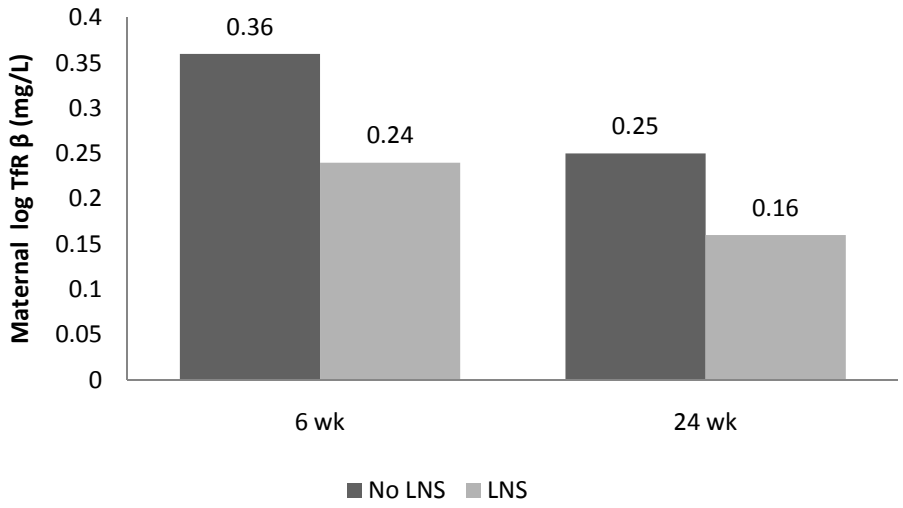

Figure 4.4 Effects of LNS among women in the MaMi subsample who recieved ARVs

Maternal ARV treatment was associated with higher maternal log TfR (suggestive of iron deficiency) at 6 and 24 weeks; but this adverse ARV effect was reduced if mothers also received LNS. Predicted maternal log TfR values (mg/L) in mothers in the ARV intervention arms from linear model of BAN study interventions maternal Hb outcomes. ARV, antiretroviral; TfR, transferrin receptors; BAN, Breastfeeding, Antiretroviral and Nutrition.

Supplemental Table 4.1 Composition of daily ration (140 g) of lipid-based nutrient supplements given to BAN Study mothers[1]

Component	Provides
Energy	746 kcal
Protein	20.8 g
Lipids	49.6 g
Iron	15 mg
Zinc	19 mg
Phosphorus	1200 mg
Selenium	75 µg
B_1 (thiamin)	1.6 mg
B_2 (riboflavin)	1.8 mg
B_3 (niacin)	20 mg equiv
B_6 (pyridoxine)	2.2 mg
B_{12} (cyanocobalamine)	2.6 µg
C (ascorbic acid)	100 mg
E (α-tocopherol)	12 mg
Folic acid	300 µg
Iodine	400 µg
Potassium	1144 mg
Magnesium	124 mg
Copper	0.30 mg
Calcium	588 mg

[1]The supplement was produced by Nutriset in Malaunay, France (www.nutriset.com) from peanut butter, vegetable fat, dried skimmed milk, dry whey, dextrinmaltose sugar and a mineral and vitamin complex. BAN, Breastfeeding Antiretroviral, and Nutrition

Supplemental Table 4.2 Longitudinal random effects model with first order autoregressive disturbance terms showing the associations between maternal Hb and infant Hb (g/dL) in 1926 BAN mother-infant pairs in the longitudinal Hb sample[1]

	Coef.	95% CI	p-value
Mom Hb	-0.04	[-0.09,0.01]	0.15
Mom Hb*Age	0.01	[0.00,0.02]	0.002
Mom Hb*Age spline	-0.02	[-0.03,-0.01]	0.004
Age	0.57	[0.43,0.71]	<0.001
Age spline	-0.67	[-0.86,-0.49]	<0.001
Female	0.25	[0.13,0.38]	<0.001
Birthweight (kg)	0.49	[0.33,0.65]	<0.001
Infant birth Hb	0.73	[0.68,0.77]	<0.001
Infant birth Hb*Age	0.07	[0.06,0.08]	<0.001
Infant birth Hb*Age spline	-0.07	[-0.07,-0.06]	<0.001
Rate of weight gain (kg/wk)	-0.27	[-0.40,-0.14]	<0.001
Rate of weight gain*Age spline	0.34	[0.31,0.38]	<0.001
Rate of weight gain*Age spline	-0.15	[-0.17,-0.12]	<0.001
Intercept	1.70	[0.59,2.82]	0.003

[1]The longitudinal model contained a spline with a knot at 9 weeks to capture the shape of infant Hb over time. BAN study interventions were tested as a potential confounders and effect-measure-modifiers, but were not included in the final model. Data from HIV-negative infants and their mothers with at least one concurrent mother infant Hb measurement and birth Hb measurement were included until 24 weeks or cessation of exclusive breastfeeding: n=341 in the mLNS-mARV arm; n=338 in the mARV arm; n=369 in the mLNS-iARV arm; n=356 in the iARV arm; n=255 in the mLNS arm; n=267 in the C arm. BAN, Breastfeeding, Antiretroviral and Nutrition; mLNS-mARV, maternal LNS/maternal ARV; mARV, maternal ARV; mLNS-iARV, maternal LNS/infant ARV; iARV, infant ARV; mLNS, maternal LNS; C, control. Hb, Hemoglobin; BAN, Breastfeeding Antiretoviral and Nutrition; LNS, lipid-based nutrient supplement; ARV, antiretroviral intervention.

Supplemental Table 4.3 Longitudinal random effects model with first order autoregressive disturbance terms showing the associations between BAN study intervention arm and maternal Hb outcomes (g/dL) in 1765 BAN mothers from 6 to 24 weeks[1]

	Coef.	95% CI	p-value
Mom Hb at 2 week	0.90	[0.88,0.93]	<0.001
mLNS-mARV	-0.41	[-0.54,-0.27]	<0.001
mARV	-0.23	[-0.37,-0.09]	0.00
mLNS	0.00	[-0.11,0.12]	0.98
mLNS-mARV*Week	0.02	[0.01,0.03]	<0.001
mLNS*Week	0.00	[-0.00,0.01]	0.62
mARV*Week	0.01	[0.00,0.02]	0.00
Week	0.01	[0.01,0.02]	<0.001
Intercept	1.08	[0.77,1.40]	<0.001

[1]A Wald test for the study intervention interactions with weeks indicated a significant effect of the interventions over time ($X^2(3) = 42.08$, $p<0.001$). Data from BAN mothers with at least one maternal Hb measurement after two weeks postpartum were included: n=569 in C; n=315 in mARV; n=573 in mLNS; n=308 in mLNS-mARV. mARV-LNS, maternal LNS/maternal ARV; mLNS, maternal LNS; mARV, maternal ARV; C, control; BAN, Breastfeeding, Antiretroviral and Nutrition; LNS, lipid-based nutrient supplement; ARV, antiretroviral drug.

Supplemental Table 4.4 Longitudinal random effects model with first order autoregressive disturbance terms showing the associations between the BAN study interventions and infant Hb (g/dL) outcomes in 1926 BAN infants[1]

	Coef.	95% CI	p-value
Infant birth Hb	0.73	[0.68,0.77]	<0.001
Birthweight	0.49	[0.34,0.65]	<0.001
Female	0.26	[0.13,0.38]	<0.001
Rate of wt gain/month	-0.28	[-0.40,-0.15]	<0.001
Age	0.72	[0.61,0.83]	<0.001
Age spline	-0.86	[-1.00,-0.72]	<0.001
Rate*Age spline	0.34	[0.31,0.38]	<0.001
Rate*Age	-0.15	[-0.17,-0.12]	<0.001
Birth Hb*Age spline	0.07	[0.06,0.08]	<0.001
Birth Hb* Age	-0.07	[-0.07,-0.06]	<0.001
mLNS-mARV	-0.09	[-0.42,0.23]	0.57
mARV	-0.11	[-0.43,0.21]	0.49
mLNS-iARV	-0.18	[-0.50,0.14]	0.26
iARV	-0.20	[-0.52,0.12]	0.22
mLNS	-0.24	[-0.59,0.11]	0.18
mLNS-mARV*Age	0.01	[-0.03,0.06]	0.53
mARV*Age	0.01	[-0.03,0.05]	0.55
mLNS-iARV*Age	-0.02	[-0.06,0.02]	0.43
iARV*Age	-0.01	[-0.05,0.03]	0.76
mLNS*Age	0.02	[-0.02,0.07]	0.32
mLNS-mARV*Age spline	-0.01	[-0.07,0.04]	0.64
mARV*Age spline	-0.01	[-0.06,0.04]	0.65
mLNS-iARV*Age spline	0.04	[-0.01,0.09]	0.11
iARV*Age spline	0.02	[-0.03,0.08]	0.38
mLNS*Age spline	-0.03	[-0.09,0.03]	0.28
Intercept	1.40	[0.40,2.40]	0.01

[1]The longitudinal model contained a spline with a knot at 9 weeks to capture the shape of infant Hb over time. Data from HIV-negative BAN infants with at least one Hb measurement after birth were included until 24 weeks or cessation of exclusive breastfeeding: n=341 in the mLNS-mARV arm; n=338 in the mARV arm; n=369 in the mLNS-iARV arm; n=356 in the iARV arm; n=255 in the mLNS arm; n=267 in the C arm. BAN, Breastfeeding, Antiretroviral and Nutrition; mLNS-mARV, maternal LNS/maternal ARV; mARV, maternal ARV; mLNS-iARV, maternal LNS/infant ARV; iARV, infant ARV; mLNS, maternal LNS; C, control. Hb, Hemoglobin; BAN, Breastfeeding Antiretoviral and Nutrition; LNS, lipid-based nutrient supplement; ARV, antiretroviral intervention.

Chapter 5: Synthesis

Overview of findings

This research examined the nutritional interrelationships of the mother-infant dyad in HIV-infected Malawian women and their exclusively breastfed infants. We used data from a large clinical trial, the Breastfeeding, Antiretrovirals and Nutrition (BAN) Study (www.thebanstudy.org), which was based in Lilongwe, Malawi, and a sub-study of BAN, the Malawi Mothers and Infants (MaMi) Study. Data for this study was obtained during the screening visit for BAN, and at BAN visits from birth to 24 weeks postpartum, corresponding with exclusive breastfeeding. Data for the MaMi study was obtained at 2 or 6 and 24 weeks. These datasets provided a unique opportunity to understand the nutritional interrelationships of the mother-infant dyad during exclusive breastfeeding. We chose to examine two important markers of maternal and infant nutritional status during this time: anthropometry and iron status. First, we explored how maternal anthropometry patterns related to infant growth. Second, we used longitudinal modeling to understand how maternal and infant Hb were related over time. Finally, we used linear models to understand how maternal and infant Hb and iron status, measured by transferrin receptors (TfR) and ferritin, were related, after accounting for inflammation and infection. The following section provides a summary and synthesis of our primary findings.

Maternal weight loss is associated with reduced length and weight gain in daughters of HIV-infected Malawian women

We aimed to understand how maternal weight changes during lactation translated into infant growth. We hypothesized that infants of mothers losing weight would have impaired growth if the mother had limited nutrient stores to mobilize. We used linear regression models to relate maternal weight loss to infant weight and length gain, adjusting for infant anthropometry at birth, maternal BMI, and covariates. We interacted maternal BMI with dichotomous weight loss to determine whether the effects of weight loss varied by initial energy stores.

We found that maternal weight loss adversely influenced growth in girls of thin mothers. This sex-specific finding was surprising, as boys are generally thought to be more susceptible to nutritional insults in utero and postpartum.[59] Previously sex differences in milk production have been observed in rhesus macaques, where mothers of sons produced higher energy and fat milk, but less milk volume compared to mothers of girls;[60] however sex differences in milk production have not been reported in humans. Given that mothers of boys lost marginally more weight than mothers of girls, perhaps mothers of boys were better able to mobilize their nutrient stores into milk production compared to mothers of girls. We are unable to test this, as we do not have measurements of milk macronutrient composition. These findings suggest that further examination of how maternal weight changes relate to breastmilk production and infant growth are warranted, especially to understand the sex differences we observed.

Maternal hemoglobin and transferrin receptors are associated with infant values during exclusive breastfeeding

We aimed to understand how maternal hemoglobin (Hb) and iron status [ferritin and transferrin receptors (TfR)] related to infant Hb longitudinally throughout exclusive breastfeeding. In a larger sample of BAN mother-infant pairs we used longitudinal regression models to relate maternal Hb to concurrently measured infant Hb, after controlling for covariates including infant birthweight, rate of weight gain, gender, and infant Hb at birth. We modeled infant age with a spline at nine weeks of age in order to capture the non-linear trend of infant Hb over time. We tested and observed significant age interactions with maternal Hb as well as the covariates rate of weight gain and infant Hb at birth.

We found that maternal Hb predicted infant Hb between 6 and 18 weeks postpartum, providing further evidence of the strong influence of maternal Hb on infant Hb over time. To provide contextual background for our primary analysis, we also examined for associations between the BAN study interventions and maternal and infant Hb over time. Though, we observed that LNS had no main effects on maternal Hb over time, the ARVs had negative but not sustained effects on maternal Hb over time. We observed no intervention effects on infant Hb over time, which has been shown elsewhere.[79]

In a subsample of BAN mother-infant pairs, the MaMi subsample, we aimed to evaluate how maternal iron status (TfR and ferritin) and Hb related to infant values during exclusive breastfeeding after accounting for infection and inflammation using multiple iron status indicators. Ferritin and TfR in particular allow for estimates of iron stores and tissue iron depletion, reppectively. We used linear regression models to relate change in maternal Hb and iron status from initial measurement to 24 weeks to change in infant value,

controlling for initial maternal and infant value, infant birthweight, infant gender and whether the measurement was obtained at 2 or 6 weeks postpartum.

We found that changes in maternal Hb and TfR were associated with change in infant value, but found no association between maternal and infant ferritin from initial measurement to 24 weeks. The relationship we observed with maternal and infant Hb, further supports the findings we observed earlier in the larger BAN sample. Though we observed no association between maternal and infant ferritin, we observed that maternal and infant TfR were related, suggesting that mothers with depleted iron stores, which manifest as increased TfR levels, may have impaired breastmilk iron levels which may adversely affect infant iron status. Finally, we evaluated whether adjusting for inflammation and infection in our mother-infant iron status and Hb models affects our findings in a sensitivity analysis and observed no marked differences in effects. Together these findings in a large sample of BAN infants and the MaMi subsample provide evidence that maternal iron status may influence infant iron status postpartum independent of the maternal influence on infant's iron endowment at birth.

Limitations and Strengths

There are several limitations to this study. First, there was much potential for selection bias, especially for the analyses with the MaMi subsample. In addition, the study requirements for participation were quite rigorous, including many long study visits, many questionnaires, blood draws and study demands; hence there was attrition. In addition, we excluded HIV-infected infants from analyses due to differences in growth and nutrient dynamics during this time. We also excluded infants after cessation of exclusive breastfeeding, as the mother was no longer the sole soure of nutrition for her growing infant at that time. For example, there were 2,363 HIV uninfected infants at birth with weight and

length data, and the sample decreased to 1,754 by 24 weeks postpartum. As such we assessed for selection bias in all of our analyses. Generally, we found that mothers and infants included in the analyses were healthier than randomized mother-infant pairs, which may have been due to excluding HIV-infected and weaned infants. Second, our results may not be generalizable to HIV infected infants or HIV unexposed infants. Based on previous research, we suspect our growth results will be generalizable to HIV unexposed infants;[25] however, we do not have a comparison group of HIV unexposed infants. Additionally, our sample of mothers and infants was quite healthy relative to other Malawians, and as such our findings may not be generalizable to other HIV exposed infants who did not have the consistent follow-up and health-care provided by BAN.

Another important consideration is whether mothers were truly exclusively breastfeeding. Because the BAN study counseled women to exclusively breastfeed for 6 months, the social desirability bias may have provoked women to report that they were still exclusively breastfeeding, when in fact they had introduced complementary foods. If infants were receiving other foods, the mother is no longer the sole supplier of nutrition to her infant; hence maternal supply will no longer be exclusively related to infant demand, and our findings may be biased. We conducted a sensitivity analysis to determine if including weaned infants changed our findings. For the weight change analysis, we included weaned infants and observed marked differences in primary associations between maternal weight loss and infant growth. For the Hb analysis, we included 378 more mother-infant Hb observations and observed that the marginally stronger primary effects of maternal Hb on infant Hb.

With the success of programs focused on preventing mother-to-child transmission of HIV, HIV-exposed-uninfected (HIV-EU) infants are a growing population that may be at

increased risk of morbidity and mortality compared to HIV unexposed infants. This study took a novel approach to determine the relationships of maternal and infant nutritional status during exclusive breastfeeding in HIV-EU infants. We believe this is the first study to examine infant iron status longitudinally during exclusive breastfeeding, accounting for maternal iron status.

This research provides evidence that maternal nutritional status is important during exclusive breastfeeding. Our findings regarding early growth of HIV-EU infants, in particular, may be generalizable to HIV unexposed infants, as there is little evidence that the growth of HIV-EU differs from HIV unexposed infants.[25] Ultimately, our findings provide guidance for designing interventions to optimize both maternal and infant nutritional status and health during pregnancy and lactation.

Significance and public health impact

This study applies a novel framework to explore the dynamic interrelationships between maternal and infant nutritional status during exclusive breastfeeding in Malawian mother-infant dyads. This study in particular adds to our understanding of growth and iron status dynamics of HIV-EU infants, an understudied population. Even though this research is in the context of HIV, our methods and findings may be applicable to non-infected populations.

There is limited understanding of how maternal weight changes shape infant growth during lactation. We utilized an innovative modeling approach to explore the complex relationships of maternal weight changes and how they affect infant growth, providing evidence of feedback loops between the mother and infant, to be examined in future research, and also highlighting significant gender differences. The current literature suggests that

maternal iron status is not an important determinant of infant iron status during exclusive breastfeeding; our study provides evidence that this is not the case and offers much insight into mother-infant iron status dynamics during this time. This is one of the first studies to examine infant iron status and Hb throughout exclusive breastfeeding, accounting for concurrently measured maternal iron status and other important determinants of infant iron status. We observed that maternal Hb and TfR are associated with infant values, providing evidence that maternal iron status does, in fact, influence breastmilk iron levels and potentially infant status.

The WHO HIV and infant feeding guidelines were recently updated and recommend exclusive breastfeeding for six months, followed by continued breastfeeding to 12 months along with provision of antiretroviral medications to the mother or the infant.[45,84] These new recommendations pose additional demands on maternal nutritional status in addition to elevated energy needs resulting from HIV infection. Given these recommendations and our findings, it is important to monitor maternal nutritional status during this time in order to ensure optimal maternal health so that she may produce optimal breastmilk for her growing infant.

In summary, this research provides evidence that maternal nutritional status is an important determinant of infant status during exclusive breastfeeding. These findings highlight that intervention targeting should focus on not only the infant, but the mother as well.

Direction for future research

The BAN and MaMi studies provided us with a novel unique opportunity to examine how maternal and infant nutrition were interrelated and interdependent during exclusive

breastfeeding in the context of HIV. In our study, we observed that maternal weight loss is associated with reduced weight and length gain in girls. Building on this research, future studies to examine the complexities of the feedback loops between mothers and infants are warranted. Moreover, further research to understand the sex differences we observed. One such study could include measurement of energy and protein content of breastmilk in mothers of boys and girls to determine if the differences observed in rhesus macaques also occur in humans.[60] We also observed that maternal Hb and TfR were associated with infant values during exclusive breastfeeding. Further analyses of these relationships are needed, especially to include additional measures of iron status markers such as zinc protoporphyrin, as well as examining different methods to adjust and account for inflammation. Future research should also include longitudinal assessment of breastmilk iron content in order to understand if changes in maternal iron status influence breastmilk iron content, and then subsequently influence infant iron status.

Further research applying a mother-infant dyad approach is urgently needed and methodological approaches to understand these relationships need to be developed. For us to be able to thoroughly understand these relationships, methods that allow us to understand how separate and shared influences of maternal and infant nutritional status affect these relationships and methods that allow for incorporation of feedback loops are essential.

Given the importance of maternal nutrition we observed, future research should be conducted in HIV infected and uninfected populations in both resource rich and resource poor settings to further understand these relationships and to determine whether there are differences depending on disease status as well as setting. Incorporation of maternal diet as well as maternal and infant morbidity into analyses would is also desirable, as these factors

may influence the associations we observed. Finally, including measures of maternal nutritional status during pregnancy may provide further insight into these complex relationships.

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