Antiretroviral Treatment Is Associated With Iron Deficiency in HIV-Infected Malawian Women That Is Mitigated With Supplementation, but Is Not Associated With Infant Iron Deficiency During 24 Weeks of Exclusive Breastfeeding

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Objective: In resource-limited settings without safe alternatives to breastfeeding, the WHO recommends exclusive breastfeeding and antiretroviral (ARV) prophylaxis. Given the high prevalence of anemia among HIV-infected women, mothers and their infants (through fetal iron accretion) may be at risk of iron deficiency. We assessed the effects of maternal micronutrient-fortified lipid-based nutrient supplements (LNS) and maternal ARV treatment or infant ARV prophylaxis on maternal and infant iron status during exclusive breastfeeding from birth to 24 weeks.

Methods: The Breastfeeding, Antiretrovirals, and Nutrition study was a randomized controlled trial conducted in Lilongwe, Malawi, from 2004 to 2010. HIV-infected mothers (CD4 >200 cells/μL) and their infants were randomly assigned to 28-week interventions: maternal LNS/maternal ARV (n = 424), maternal LNS/infant ARV (n = 426), maternal LNS (n = 334), maternal ARV (n = 425), infant ARV (n = 426), or control (n = 334). Longitudinal models tested intervention effects on hemoglobin (Hb). In a subsample (n = 537) with multiple iron indicators, intervention effects on Hb, transferrin receptors (TfR), and ferritin were tested with linear and Poisson regression.

Results: In longitudinal models, LNS effects on maternal and infant Hb were minimal. In subsample mothers, maternal ARVs were associated with tissue iron depletion (TfR >8.3 mg/L) (risk ratio: 3.1, $P < 0.01$), but not in ARV-treated mothers receiving LNS ($P = 0.17$). LNS without ARVs was not associated with iron deficiency or anemia ($P > 0.1$). In subsample infants, interventions were not associated with impaired iron status (all $P > 0.1$).

Conclusions: Maternal ARV treatment with protease inhibitors is associated with maternal tissue iron depletion; but LNS mitigates adverse effects. ARVs do not seem to influence infant iron status; however, extended use needs to be evaluated.

Key Words: iron, breastfeeding, lipid-based nutrient supplement, antiretrovirals, maternal, infant

(*J Acquir Immune Defic Syndr* 2015;69:319–328)

Received for publication September 2, 2014; accepted January 21, 2015.

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The University of North Carolina received grant support from Abbott Laboratories and GlaxoSmithKline.

Presented at Experimental Biology April 10, 2011, Washington, DC, and the International Lipid Based Nutrient Supplement Symposia April 8, 2011, Washington, DC.

C.M.v.d.H. received grant support from Abbott Laboratories and GlaxoSmithKline. The remaining authors have no conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jaids.com).

M.E.B., C.S.C., D.J.J., A.P.K., C.M.v.d.H., and L.S.A. contributed to the trial design. D.K., C.S.C., Z.K., G.T., and S.R.E. contributed to data collection. L.H.A. and S.S.-F. contributed to biomarker assays for the subsample. E.M.W., V.L.F., and L.S.A. contributed to interpretation of data analysis. E.M.W. performed the data analysis. E.M.W. wrote the article. E.M.W. had primary responsibility for final content. All authors reviewed major revisions and contributed to the intellectual content of article.

This trial was registered at http://www.clinicaltrials.gov NCT00164736.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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INTRODUCTION

In resource-poor settings, the WHO recommends that HIV-infected women exclusively breastfeed for 6 months and continue breastfeeding to 12 months.[1] In this population, antiretrovirals (ARVs) are provided to the mother or infant to prevent mother-to-child transmission of HIV (PMTCT) if replacement feedings are not acceptable, feasible, affordable, sustainable, and safe.[1] HIV-infected women are at risk of impaired iron status during pregnancy and postpartum due to heightened iron demands in this period coupled with the demands of the HIV infection.[2–5]

Given the strong influence of maternal iron status on infants' iron endowment at birth and thus subsequent iron status,[6] infants born to HIV-infected mothers are at high risk of iron deficiency.[7,8]

Some prenatally administered ARVs, especially zidovudine, are associated with maternal anemia[9] and severe infant anemia postpartum.[10] This is in contrast to findings in nonpregnant adult populations, where initiation of highly active antiretroviral therapy (HAART) is associated with increases in hemoglobin (Hb).[11–13] Although some studies have shown that single-dose infant nevirapine may have transient effects on infant iron status,[14,15] extended infant nevirapine regimens do not seem to influence short- and long-term risk of anemia.[16,17] However, data regarding the effects of extended postpartum PMTCT regimens on maternal and infant iron status are limited and no studies to date have reported results among mothers also receiving nutritional supplementation.

The Breastfeeding, Antiretrovirals, and Nutrition (BAN) study was a randomized controlled trial designed to test interventions for PMTCT.[17] Mother–infant pairs were randomized with a two-by-three factorial design to one of six 28-week treatment assignments: 3 ARV groups (maternal ARV, infant nevirapine or no extended postnatal ARV) and 2 maternal nutritional intervention groups [lipid-based nutrient supplements (LNS) or no LNS]. Previously, we reported that the proportion of low Hb (grade 3 or 4 adverse events) in both mothers and infants did not differ by ARV group; however, this analysis did not evaluate effects of LNS.[17] This secondary analysis explores the effects of the 6 treatments on (1) maternal and infant Hb longitudinally during exclusive breastfeeding and in a subsample with multiple iron indicators and (2) maternal and infant ferritin, transferrin receptors (TfR), and Hb adjusted for the acute-phase response. We hypothesized that ARVs would be associated with worsening maternal and infant iron indicators, and that LNS would be associated with improved maternal iron indicators. We did not expect to observe LNS effects in the infants because previous evidence suggests that maternal iron supplementation during breastfeeding does not influence infant iron status.[18]

METHODS

Participants

Data are from the BAN Study (clinicaltrials.gov number NCT00164736), conducted from 2004 to 2010, whose design[19] and primary intervention findings have been previously reported.[17,20–22] Briefly, HIV-1–infected pregnant women (n = 3572) were recruited from antenatal clinics in Lilongwe, Malawi. Primary eligibility criteria for initial enrollment included gestational age ≤30 weeks, CD4 ≥250 cells per microliter (CD4 ≥200 cells per microliter before July 2006), and Hb ≥70 g/L. After delivery, secondary eligibility criteria for randomization included infant birth weight ≥2 kg, and no previous ARV use.[19] Infants diagnosed with HIV-1 within 2 weeks of delivery were withdrawn from the study and referred for care.[17]

Ethical Approval

The Malawi National Health Science Research Committee and the institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention provided ethical approval for the study, and the institutional review board at the University of California, Davis approved the laboratory analyses at the Western Human Nutrition Research Center. Written informed consent was obtained from all study mothers.

Randomization

At delivery, mother–infant dyads (n = 2369) were randomized to one of six 28-week treatment arms: maternal LNS/maternal ARV (mLNS-mARV), maternal LNS/infant ARV (mLNS-iARV), maternal LNS only (mLNS), maternal ARV only (mARV), infant ARV only (iARV), or control.

Study Interventions

All mothers received daily iron–folic acid supplementation containing 40 mg elemental iron and 0.25 mg folic acid from the initial screening to 1 week postpartum. All mothers and infants also received an intrapartum single dose of nevirapine and twice-daily zidovudine and lamivudine for 7 days after delivery.[14]

Mothers assigned to the nutritional intervention were given 140 g of LNS per day, providing 746 kcal, 20.8 g protein and 15 mg of elemental iron (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A651).[22] To prevent sharing of LNS, all mothers were provided maize for family consumption.[22] The maternal ARV regimen initially consisted of zidovudine, lamivudine, and nevirapine. Nelfinavir replaced nevirapine after the first 39 women were randomized, and lopinavir/ritonavir replaced nelfinavir shortly thereafter, with most (>75%) mothers randomized to ARV receiving zidovudine/lamivudine/lopinavir–ritonavir. Infants randomized to ARV received daily oral nevirapine (details previously reported).[17] Starting on June 13, 2006, cotrimoxazole prophylaxis (CPT) (240 mg once daily) was provided to all infants aged >6 weeks and initiated for all mothers with a CD4 <500 cells per microliter in pregnancy[23]; in this report, 68% and 43% of infants and mothers received CPT, respectively. In March 2008, the data safety monitoring board at the National Institute of Allergy and Infectious Diseases reviewed data from the enrolled 1857 mother–infant pairs and observed that HIV transmission through breast milk was higher in the no-drug study arms.[24] Enrollment was halted in these groups, and mothers in these arms were allowed to choose to switch to the maternal or infant ARVs, or remain in the control group.[24]

Mothers were provided additional iron supplementation as clinically indicated or with ≥moderate anemia (Hb ≤84 g/L[25]). Records of additional iron supplementation were abstracted from medication, severe adverse event, and morbidity forms, linked to concomitant study visit(s). Few participants (<3%) were given supplemental iron.

Study visits were conducted at the BAN Study clinic in Lilongwe, Malawi, at birth, 1, 2, 4, 6, 8, 12, 18, 21, and 24 weeks of postpartum. This analysis includes data from 0 to 24

weeks, when mothers were provided with intensive counseling to exclusively breastfeed.[26,27] Maternal Hb was measured at prenatal screening and maternal and infant Hb (g/L) was measured at birth, 2, 6, 12, 18, and 24 weeks of postpartum from venous blood samples with a Beckman Coulter AcT or AcT 5-part Differential Analyzer (Beckman Coulter, Fullerton, CA). Infant HIV status was tested with Amplicor 1.5 DNA polymerase chain reaction. Measurements included maternal and infant weights with regularly calibrated Tanita digital scales, infant length with a recumbent length board, and maternal height with a wall-mounted stadiometer. At screening, maternal report of parity, marital status, and education was obtained. Exclusive breastfeeding was obtained by maternal report.

Plasma for additional biomarker assays was separated from red blood cells, aliquoted to 1-mL polypropylene storage tubes, and stored at −70°C. Briefly, subsample laboratory analyses used plasma obtained at either 2 or 6 weeks, as many infants had insufficient plasma at 2 weeks, and 24 weeks postpartum. Transferrin receptors (TfR) and inflammatory marker [C-reactive protein (CRP) and α-1-acid glycoprotein (AGP)] concentrations were measured using Cobas Integra 400 (Roche Diagnostics, Indianapolis, IN).[28] Ferritin concentrations were measured with IRMA Ferritin Coat a Count radioimmunoassay (Siemens Health Care Diagnostics Inc., Plainfield, IN).[28]

Statistical Analysis

The primary outcomes for this analysis are maternal and infant Hb, ferritin, and TfR. The primary independent variables are the study intervention arms. For the maternal outcomes, 4 intervention groups were evaluated; mothers of infants receiving ARVs (mLNS-iARV and iARV) were included in the mLNS and control groups, respectively (see Figure S1, Supplemental Digital Content, http://links.lww.com/QAI/A651). Mother–infant pairs were excluded from both analysis samples if the infant was a multiple birth (n = 49), which may influence fetal iron accretion, or HIV-infected from 2 to 24 weeks (n = 57), which may influence iron mobilization and interpretation of iron indicators.[29] Observations after breastfeeding cessation (n = 277) were dropped in longitudinal analyses, as cessation would influence maternal nutritional status and the infant would no longer be exposed to maternal ARVs through breast milk. Of the 2369 randomized dyads, 1765 and 1927 were included in the longitudinal maternal and infant analytic samples, respectively (see Figure S2, Supplemental Digital Content, http://links.lww.com/QAI/A651). Subsample dyads (n = 537) were selected with equal representation from the LNS and no-LNS groups, prioritizing those with anthropometry and dietary data and excluding multiple births and HIV-positive infants (see Figure S3, Supplemental Digital Content, http://links.lww.com/QAI/A651).[28] Characteristics of included and excluded dyads in each analytic sample were compared for similarity using *t* tests and nonparametric tests for normally distributed and skewed continuous variables, respectively. Similar tests compared characteristics of those only in the longitudinal sample versus the subsample. Because a small number of mothers in this analysis received additional iron supplementation, we evaluated whether supplementation varied by treatment arm and observed no differences across treatment arms at each visit (all $P > 0.19$; data not shown). STATA 12.0 (College Station, TX) was used for all statistical analyses. An α of 0.05 denoted statistical significance.

Infants were included in the longitudinal analyses if they had birth weight and Hb at birth, and at least one other Hb measurement. Mothers were included in the longitudinal analysis if they had Hb at 2-week postpartum and at least one other measurement. Because of hematologic dilution at delivery and inflammation of parturition,[30] which would distort initial measurements, maternal models used the 2-week Hb value as baseline. Longitudinal random-effects models with first-order autoregressive disturbance terms were used to evaluate the effect of the study interventions on (1) maternal Hb from 6- to 24-week postpartum and (2) infant Hb from 2 to 24 weeks, adjusting for week and initial Hb.[31] Infant models were further adjusted for birth weight, growth rate, and sex. In infant models, age was modeled in weeks and included a knot at 9 weeks of age to capture the shape of the infant Hb curve over time. To determine whether intervention effects varied with infant age, interactions of week with covariates were evaluated using Wald tests for joint significances ($\alpha = 0.1$[32]) and significant interaction terms were retained.

In the subsample, we accounted for the acute-phase response and minimized the effects of the infection (such as malaria and HIV) and inflammation [elevated CRP (>5 mg/L) and AGP (>1 g/L)] on TfR, Hb, and ferritin.[28] Stage of inflammation [healthy (normal CRP and AGP), incubation (elevated CRP), early convalescence (CRP and AGP elevated), late convalescence (elevated AGP)], and stage-specific correction factors were determined.[28,33,34] Plasma ferritin and TfR had non-Gaussian distributions and were log transformed for analyses. Linear regression was used to evaluate the effects of study interventions on maternal and infant iron status (ferritin and TfR) and Hb. Modified Poisson regression with robust variance estimators was used to estimate relative risk of maternal and infant deficiency or anemia at 24 weeks.[35] For all models, sensitivity analyses were conducted to evaluate whether inclusion of an indicator variable for maternal iron supplementation or CPT influenced the study intervention effects.

RESULTS

Maternal

Most baseline maternal characteristics were well balanced by study arm for the longitudinal sample and the subsample (Table 1). A smaller proportion of control mothers were married or educated beyond primary school in the longitudinal sample (Table 1). Compared with randomized mothers not included in this analysis, mothers in both analytic samples were older, more educated, had higher pregnancy Hb, and lower body mass index at delivery. Compared with mothers only in the longitudinal sample, mothers in the subsample had lower body mass index at delivery (data available in online supplemental materials, http://links.lww.com/QAI/A651).

TABLE 1. Baseline Characteristics by Study Intervention Group in HIV-Infected Malawian Mothers and Their HIV-Exposed Infants in the BAN Study, Malawi, 2004–2010*

	mARV-mLNS	mARV	mLNS-iARV	iARV	mLNS	Control	P†
Maternal							
Longitudinal sample	n = 308	n = 315			n = 573	n = 569	
Age (years)	26 (23–30)	26 (23–30)			26 (23–30)	26 (23–29)	0.62
Education beyond primary school (%)	114 (37.0)	119 (37.8)			224 (39.1)	176 (30.9)	0.02
Married (%)	523 (91.9)	537 (93.7)			291 (94.5)	278 (88.3)	0.02
Maternal body mass index (kg/m^2)	22.8 (21.5–25.0)	23.3 (21.6–25.7)			23.3 (21.4–25.2)	23.2 (21.6–25.3)	0.30
Pregnancy CD4 (cells/μL)	442 (329–581)	438 (332–567)			442 (328–599)	438 (338–568)	0.91
CD4 <350 cells/μL (%)	94 (30.5)	88 (27.9)			169 (29.5)	158 (27.8)	0.80
Pregnancy Hb (g/L)	110 (101–117)	110 (101–117)			108 (100–116)	109 (101–117)	0.29
Anemic during pregnancy (%)‡	298 (52.4)	308 (53.8)			148 (48.1)	155 (49.2)	0.13
Primiparous (%)	30 (9.7)	37 (11.8)			67 (11.7)	53 (9.3)	0.67
Subsample	n = 90	n = 85			n = 185	n = 177	
Age (years)	25 (23–30)	27 (24–29)			26 (22–30)	25 (23–30)	0.58
Education beyond primary school (%)	30 (33.3)	31 (36.5)			72 (38.9)	65 (36.7)	0.85
Married (%)	83 (92.2)	76 (89.4)			167 (90.3)	160 (90.4)	0.94
Maternal body mass index (kg/m^2)	22.7 (21.5–24.6)	22.8 (21.1–25.1)			22.9 (21.2–24.6)	22.9 (21.5–25.0)	0.75
Pregnancy CD4 (cells per μL)	414 (319–584)	468 (298–590)			448 (328–596)	424 (327–570)	0.88
CD4 <350 cells/μL (%)	28 (31.1)	26 (30.6)			54 (29.2)	53 (29.9)	0.99
Pregnancy Hb (g/L)	110 (101–117)	109 (100–116)			107 (99–116)	109 (100–117)	0.52
Anemic during pregnancy (%)‡	43 (47.8)	44 (51.8)			104 (56.2)	95 (53.7)	0.61
Primiparous (%)	8 (8.9)	7 (8.2)			19 (10.3)	20 (11.3)	0.99
Infant							
Longitudinal sample	n = 341	n = 339	n = 369	n = 356	n = 255	n = 267	
Female (%)	170 (49.9)	168 (49.6)	182 (49.3)	178 (50.0)	130 (51.0)	122 (45.7)	0.88
Birth weight (kg)	3.0 (2.8–3.3)	3.0 (2.7–3.3)	3.0 (2.8–3.3)	3.0 (2.8–3.3)	3.0 (2.75–3.3)	3.0 (2.7–3.3)	0.91
Low birth weight (%)§	16 (4.7)	29 (8.6)	19 (5.2)	24 (6.7)	16 (6.3)	16 (6.0)	0.39
Birth length (cm)	48.0 (47.0–49.8)	48.0 (47.0–49.9)	48.0 (47.0–49.2)	48.0 (47.0–50.0)	48.2 (47.0–50.0)	48.0 (47.0–49.0)	0.30
Hb (g/L)	175 (163–189)	175 (161–187)	174 (162–187)	175 (163–188)	174 (161–187)	177 (160–188)	0.94
Subsample	n = 90	n = 85	n = 107	n = 111	n = 77	n = 66	
Female (%)	41 (45.6)	37 (45.3)	56 (52.3)	53 (47.8)	42 (54.6)	29 (43.9)	0.63
Birth weight (kg)	3.0 (2.8–3.3)	3.1 (2.9–3.3)	3.0 (2.8–3.3)	3.0 (2.8–3.3)	3.0 (2.7–3.4)	3.0 (2.7–3.3)	0.65
Low birth weight (%)§	6 (6.7)	3 (3.5)	5 (4.7)	4 (3.6)	5 (6.5)	2 (3.0)	0.83
Birth length (cm)	48.3 (47.0–49.5)	48.2 (47.0–49.5)	48.0 (47.0–49.8)	48.1 (47.0–49.8)	48.2 (47.0–49.4)	48.0 (47.0–49.4)	0.77
Hb (g/L)	173 (160–185)	171 (160–188)	171 (158–187)	176 (161–187)	174 (159–190)	178 (165–191)	0.47

Data are median (interquartile range) and n (%). Study intervention arms: study intervention: maternal LNS/maternal ARV (mLNS-mARV), maternal LNS/infant ARV (mLNS-iARV), maternal LNS (mLNS), maternal ARV (mARV), infant ARV (iARV), or control.

*For maternal data, iARV and iARV-mLNS were included in the control and mLNS groups, respectively.

†*P* values based on Kruskal–Wallis test for continuous variables and Fisher exact test for binary variables.

‡Hb <110 g/L.

§Low birth weight <2.5 kg.

Maternal Hb increased from baseline to 24 weeks (Fig. 1) and thus prevalence of anemia (Hb <120 g/L, unadjusted for inflammation) decreased. In subsample mothers, prevalence of tissue iron depletion (TfR >8.3 mg/L) declined, whereas prevalence of depleted iron stores (ferritin <15 ng/mL) remained somewhat stable (Table 2).

In the longitudinal analysis, mLNS had no effects on maternal Hb (Table 3). There were some transient effects of maternal ARVs on maternal Hb (Table 3). Based on linear combinations of the beta coefficients for the intervention groups and weeks, maternal Hb was significantly lower at 6 and 12 weeks, but not at subsequent observations, in mothers who received mLNS-mARV [6 weeks—β: −3.21 g/L [95% confidence interval (CI): −4.46 to −1.94], $P < 0.001$; 12 weeks—β: −1.88 g/L (95% CI: −2.91 to −0.85), $P < 0.001$] or mARV [6 weeks—β: −2.11 g/L (95% CI: −3.35 to −0.87), $P = 0.001$; 12 weeks—β: −1.47g/L (95% CI: −2.50 to −0.45), $P = 0.005$] (Table 3).

In subsample mothers, mLNS was not associated with maternal Hb, TfR, or ferritin, compared with control (all $P > 0.1$) (see Table S2, Supplemental Digital Content, http://links.lww.com/QAI/A651). mARV and mLNS-mARV were associated with lower Hb at 6 weeks and higher TfR at 6 and 24 weeks (indicative of worsening iron status), but

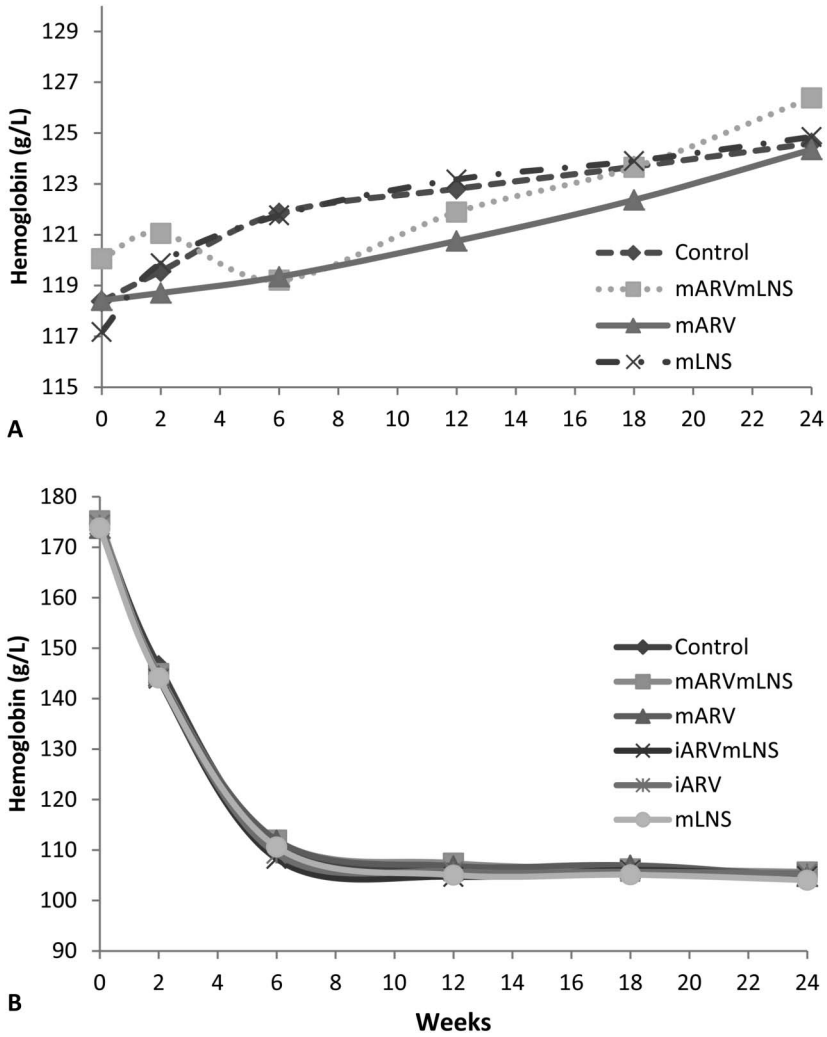

FIGURE 1. A, Mean maternal Hb (g/L) values according to BAN study arm from birth to 24 weeks among HIV-infected Malawian mothers in the longitudinal sample (n = 569 in the control arm; n = 315 in the mARV arm; n = 573 in the mLNS arm; n = 308 in the maternal ARV-LNS arm). B, Mean infant Hb (g/L) values according to BAN study arm from birth to 24 weeks among HIV-exposed Malawian infants in the longitudinal sample. BAN study, Malawi, 2004–2010 (n = 341 in the mLNS-mARV arm; n = 339 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 control arm). mLNS-mARV, maternal LNS/maternal ARV; mARV, maternal ARV; mLNS-iARV, maternal LNS/infant ARV; iARV, infant ARV; mLNS, maternal LNS; C, control.

were not associated with ferritin (see Table S2, Supplemental Digital Content, http://links.lww.com/QAI/A651). Although the interventions were not associated with risk of maternal anemia (Hb <120 g/L) or depleted iron stores (ferritin <15 ng/mL) at 24 weeks, mARV was associated with increased risk of tissue iron depletion (TfR >8.3 mg/L) but only in ARV-treated mothers who did not receive LNS (Table 4). Although continuous TfR values were higher in mothers in the mLNS-mARV group at 24 weeks (see Table S2, Supplemental Digital Content, http://links.lww.com/QAI/A651), mothers in this group were not at higher risk of tissue iron depletion compared with controls. In sensitivity analyses, adjustment for additional maternal iron supplementation and CPT had no or negligible changes on intervention effects (data not shown).

Infant

Baseline infant characteristics were well balanced across the study arms (Table 1). Compared with other randomized dyads, infants in both analytic samples were heavier at birth and more likely to be in the iARV arms (data available in online supplemental materials, http://links.lww.com/QAI/A651).

Mean infant Hb followed the normal pattern of decline from a high of above 170 g/L at birth to a nadir at about 8 weeks, as senescent fetal Hb lyses, with a subsequent slight increase thereafter (Fig. 1).[29] In subsample infants, ferritin values declined from initial measurement to 24 weeks, as values normalized after erythrocyte breakdown, whereas TfR increased. Few infants had impaired iron status based on measurements at birth or 2 or 6 weeks, but at 24 weeks, worsening iron status was apparent (Table 2).

In the longitudinal analytic sample, minimal intervention effects were observed on infant Hb (see Table S3, Supplemental Digital Content, http://links.lww.com/QAI/A651). Based on linear combinations of the beta coefficients for each intervention arm and age, mLNS-iARV was associated with 2.8 g/L ($P = 0.03$) lower Hb at 6 weeks; but was not associated with lower Hb values at later visits [12 weeks—β: −2.51 g/L (95% CI: −5.16 to 0.15), $P = 0.06$; 18 weeks—β: −0.97 g/L (95% CI: −3.44 to 1.51), $P = 0.44$; 24 weeks—β: 0.57 g/L (95% CI: −2.25 to 3.39), $P = 0.7$] (see Table S3, Supplemental Digital Content, http://links.lww.com/QAI/A651). In the subsample infants where Hb was corrected for

TABLE 2. Infection and Inflammation-Adjusted Iron Status Indicators in HIV-Infected Malawian Mothers and HIV-Exposed Infants in the BAN Study Subsample, 2004–2010*

	Initial Measurement		
	2 wk	6 wk	24 wk
Maternal			
Hb (g/L)†	125.5 ± 16.1 (360)	122.1 ± 13.4 (170)	125.5 ± 11.5 (534)
Low (%)‡	35.3	38.8	29.6
Ferritin (ng/mL)†	29.98 ± 37.26 (362)	43.09 ± 63.69 (170)	32.38 ± 43.08 (536)
Deficient (%)‡	39.2	32.4	33.4
TfR (mg/L)†	5.69 ± 2.82 (362)	5.67 ± 3.50 (170)	4.86 ± 2.27 (535)
Deficient (%)‡	12.7	15.9	6.9
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/L)†	143.0 ± 17.8 (351)	111.4 ± 12.8 (163)	104.7 ± 11.0 (526)
Low (%)§	‖	‖	51.7
Ferritin (ng/mL)†	462.68 ± 338.66 (359)	314.67 ± 228.92 (167)	42.34 ± 102.80 (533)
Deficient (%)§	2.0	6.0	31.0
TfR (mg/L)†	3.53 ± 1.90 (360)	3.35 ± 1.91 (165)	6.64 ± 3.33 (531)
Deficient (%)§	1.7	3.0	21.9
Elevated inflammatory markers			
CRP >5 mg/L (%)	8.9	16.2	28.5
AGP >1 g/L (%)	8.6	16.1	47.0

*Adjusted for inflammation by using group-specific correction factors estimated from ratios of medians for the various iron indicators.[34,35]
†Values are mean ± SD (n).
‡Low maternal Hb <120 g/L,[2] abnormal ferritin <15 ng/mL, abnormal TfR >8.3 mg/L.
§Low infant Hb was defined as <105 g/L at 24 weeks.[25] Abnormal infant TfR >8.3 mg/L and ferritin <30 ng/mL at 2/6 weeks and <12 ng/mL at 24 weeks.
‖Cutpoints for low infant Hb are not available <3 months of age.
AGP, plasma α_1-acid glycoprotein; CRP, C-reactive protein; Hb, hemoglobin; TfR, plasma soluble transferrin receptor.

inflammation, however, some intervention effects on continuous Hb values were observed. Although mLNS had no effect on Hb at 2 or 6 weeks, it was associated with lower infant Hb at 24 weeks (see Table S2, Supplemental Digital Content, http://links.lww.com/QAI/A651). Compared with controls, mLNS-iARV and iARV were associated with lower Hb values at 2 and 24 weeks (β range: 4–5 g/L), whereas a similar but nonsignificant trend was observed at 6 weeks (β range: 4.3–5.3 g/L) (see Table S2, Supplemental Digital Content, http://links.lww.com/QAI/A651).

TABLE 3. Longitudinal Random-Effects Model Showing the Associations Between the Study Intervention Arms and Maternal Hb Outcomes (G/L) from 6 to 24 Weeks in 1765 HIV-Infected Malawian Mothers in the BAN Study, 2004–2010*

	Coefficient	95% Confidence Interval	*P*
Maternal Hb at 2 wk	0.51	0.49 to 0.54	<0.001
Week	0.15	0.10 to 0.20	<0.001
mLNS-mARV	−4.49	−6.17 to −2.80	<0.001
mARV	−2.70	−4.38 to −1.03	0.002
mLNS	−0.11	−1.52 to 1.30	0.88
mLNS-mARV × wk	0.22	0.13 to 0.31	<0.001
mARV × wk	0.10	0.01 to 0.19	0.03
mLNS × wk	0.01	−0.07 to 0.09	0.80
Intercept	59.39	56.44 to 62.34	<0.001

*The control arm was the reference group in this longitudinal model. A Wald test for the study intervention interactions with weeks indicated a significant effect of the interventions over time ($\chi^2(3) = 27.47$, $P < 0.001$). Data from BAN mothers with at least 1 maternal Hb measurement after two-week postpartum were included: n = 569 in control; n = 316 in mARV; n = 573 in mLNS; n = 308 in mLNS-mARV.
Hb, hemoglobin; mARV-LNS, maternal LNS/maternal ARV; mLNS, maternal LNS; mARV, maternal ARV; wk, week.

At 24 weeks, mLNS was associated with lower infant ferritin values (see Table S2, Supplemental Digital Content, http://links.lww.com/QAI/A651). mLNS and mLNS-mARV were associated with higher infant TfR at 2 weeks, but not at other times. The interventions were not associated with risk of infant iron deficiency (inflammation-adjusted ferritin <12 ng/mL; TfR >8.3 mg/L) or anemia (inflammation-adjusted Hb <105 g/L) at 24 weeks (Table 4). In sensitivity analyses, adjustment for additional maternal iron supplementation or infant CPT did not influence intervention effects on infant Hb or iron status (data not shown).

DISCUSSION

To our knowledge, this is the first study to investigate effects of extended ARVs coupled with maternal nutrition supplementation on maternal and infant iron status during exclusive breastfeeding. Our results suggest that maternal ARV therapy and infant nevirapine prophylaxis were associated with worsening of some maternal and infant iron status indicators. Most notably, maternal ARVs were associated

TABLE 4. Associations Between the Study Interventions and Risk of Iron Deficiency or Anemia at 24 Weeks in HIV-Infected Malawian Mothers and Their HIV-Exposed Infants in the BAN Study Subsample, 2004–2010*

	Iron Deficiency						Anemia		
	High TfR			Low Ferritin			Low Hb		
	RR	95% CI	*P*	RR	95% CI	*P*	RR	95% CI	*P*
Maternal†		n = 535			n = 536			n = 534	
mARV-mLNS	1.96	0.76 to 5.04	0.17	1.31	0.95 to 1.81	0.10	1.00	0.67 to 1.49	0.99
mARV	3.11	1.32 to 7.32	0.01	0.99	0.68 to 1.44	0.94	1.14	0.78 to 1.68	0.49
mLNS	1.08	0.42 to 2.73	0.88	0.96	0.71 to 1.30	0.80	1.01	0.73 to 1.40	0.95
Infant‡		n = 531			n = 533			n = 526	
Female	0.51	0.36 to 0.72	<0.001	0.53	0.41 to 0.70	<0.001	0.78	0.66 to 0.92	0.003
Birth weight	0.45	0.29 to 0.69	<0.001	0.53	0.38 to 0.74	<0.001	0.58	0.46 to 0.72	<0.001
mLNS-mARV	1.24	0.65 to 2.36	0.52	1.02	0.62 to 1.69	0.93	1.01	0.72 to 1.42	0.94
mARV	1.40	0.75 to 2.63	0.29	0.96	0.57 to 1.63	0.89	1.06	0.76 to 1.48	0.75
mLNS-iARV	1.40	0.76 to 2.58	0.28	1.44	0.92 to 2.26	0.11	1.19	0.88 to 1.62	0.27
iARV	1.38	0.74 to 2.57	0.31	1.36	0.85 to 2.15	0.20	1.29	0.95 to 1.74	0.10
mLNS	0.94	0.45 to 1.97	0.88	1.45	0.89 to 2.38	0.14	1.31	0.95 to 1.80	0.10

*Risk ratios were estimated with modified Poisson regression with robust variance estimators. The control arm was the reference group. TfR, ferritin, and Hb values were adjusted for inflammation.[28,29]

†Impaired maternal iron status: low Hb <120 g/L, low ferritin <15 ng/mL, high TfR >8.3 mg/L.

‡Impaired infant iron status: low Hb <105 g/L,[25] low ferritin <12 ng/mL, and high TfR >8.3 mg/L.

Hb, hemoglobin; iARV, infant ARV; MaMi, Malawi Mothers and Infants; mARV, maternal ARV; mLNS, maternal LNS; TfR, transferrin receptors.

with a 3-fold risk of tissue iron depletion, but this risk was mitigated by mLNS, where adverse effects on tissue iron depletion (TfR levels) were reduced. Although the mLNS contained only 15 mg of iron, this amount was sufficient to mitigate some of the adverse ARV effects on maternal tissue iron status. Interestingly, the interventions did not impact risk of maternal iron store depletion (low ferritin) or anemia. Tissue iron depletion reflects more severe iron deficiency, as TfR levels increase when ferritin values become subnormal (eg, iron stores are depleted); therefore, populations can have depleted iron stores before tissue iron depletion is apparent. Maternal supplementation had few clinically meaningful or sustained effects on infant TfR, Hb, or ferritin, and neither the mLNS nor maternal or infant ARVs were associated with increased risk of infant iron deficiency or anemia at 24 weeks.

Several randomized trials and cohort studies have demonstrated adverse effects of ARVs on maternal or infant iron status, particularly zidovudine-containing regimens[9,14,16,36–45]; however, none, other than ours,[17] evaluated extended postnatal ARV regimens coupled with a nutritional supplement. Furthermore, none of these trials evaluated multiple iron status indicators, nor did they account for the effect of inflammation and infection on iron status. In HIV-affected populations, anemia of inflammation is common, defined as the presence of inflammation without iron deficiency anemia, and initiation of ARV therapy may differentially impact indicators of iron status (increase TfR and Hb); thus, measurement of the acute-phase response to correct iron indicators for concurrent inflammation/infection is recommended for assessment of iron status.[46] With inflammation and infection, iron status indicators are distorted; plasma ferritin levels are falsely elevated and do not reflect body iron stores, whereas Hb levels are lowered.[46] TfR may be increased with iron deficiency anemia in the presence of inflammation[46]; but it is believed to be less sensitive to the effects of anemia of inflammation/infection than other indicators. Previously, in BAN, we observed associations between inflammatory markers and TfR, ferritin, and Hb[28]; therefore, in our subsample analyses, we used methods recommended by Thurnham et al to mitigate the effects of the acute-phase response on iron status indicators.[34]

This is the first study to use longitudinal data with a true control group to report hematologic effects of maternal ARV exposure on multiple postpartum iron indicators. In a cohort study of pregnant HIV-infected women in Thailand, anemia (Hb <94 g/L) was significantly associated with HAART use compared with ARVs for PMTCT with both regimens containing zidovudine ($P = 0.02$).[9] Another cohort study in Italy compared hematologic outcomes of ARVs in 3 groups: (1) women receiving zidovudine-based HAART from conception, (2) women starting zidovudine-based HAART during pregnancy, and (3) women receiving zidovudine-free HAART from conception.[36] Women receiving zidovudine-free HAART had a greater decrease in Hb from baseline to 36-week gestation compared with women who started a zidovudine-containing HAART regimen during pregnancy (-20.3 ± 11.9 g/L vs. -13.6 ± 12.0 g/L, $P = 0.04$).[36] Together, these studies suggest that prenatal maternal Hb may be compromised by some ARV regimens, but there is insufficient evidence that prenatal ARVs always lead to anemia. We did not observe sustained adverse effects of postpartum ARVs on maternal Hb. This may reflect use of different ARV regimens, sample differences (BAN excluded mothers with Hb <70 g/L), or prenatal/postpartum period differences. Although there is evidence that initiation of HAART (with various combinations of drugs) is associated with increased Hb and/or improved anemia in nonpregnant populations,[11–13] these results may not be comparable with

pregnant/postpartum populations due to heightened iron demands in this period or to relatively healthy HIV-infected pregnant/postpartum women receiving ARVs.

Several studies have indicated that ARV regimens are associated with adverse effects on infant Hb,[10,37–45] whereas 1 study has shown some positive effects.[15] Infants who received a short-course regimen, including maternal peripartum nevirapine and/or infant zidovudine and/or nevirapine, had higher Hb from birth to 6 months and lower prevalence of deficiency at 6 months.[15] However, no differences in deficiency prevalence were observed beyond 6 months.[15] Adverse and often transient hematologic effects of zidovudine-containing ARV regimens during pregnancy have been reported in numerous cohorts,[10,38–43] with many of these cohorts reporting severe anemia in HAART-exposed infants.[10,41] In addition, postnatal zidovudine exposure has been associated with lower infant Hb and/or increased infant anemia[10,37,41,43,44] and may have long-lasting adverse effects on infant Hb up to 18 months postpartum.[45] Similar to previous reports, we observed transient effects of maternal and infant ARVs on some infant Hb and TfR; however, we did not observe increased anemia or iron deficiency, which may be attributable to sample differences related to BAN inclusion criteria, type and timing of initiation of ARVs (infants in BAN were not exposed to ARVs in utero), and close follow-up, as well as our report of additional iron indicators and adjustment for inflammation.

While no prior studies reported effects of maternal LNS on maternal or infant iron status, 1 study examined the effects of multivitamin supplementation during pregnancy and postpartum on maternal and infant iron status in the context of HIV.[36] In a Tanzanian cohort of ARV-naive and predominately anemic HIV-infected women who received standard iron and folic acid supplementation during pregnancy (containing 120 mg iron and 5 mg folic acid), maternal multivitamin supplementation, containing no iron, starting prenatally and continuing through lactation was associated with 8.8 g/L ($P = 0.0002$) higher maternal Hb at $\leq$70 days postpartum compared with placebo.[47] The authors speculated that vitamins in the supplement, especially vitamin C, may have contributed to better iron status and enhanced absorption of dietary iron.[47] Although we did not observe effects of this magnitude in our mothers, who started receiving supplementation postpartum, mLNS was associated with reduced tissue iron depletion in mother's receiving ARVs. Infant Hb outcomes in this Tanzanian population were not significantly different between supplementation groups at 6 months, infant Hb was 3.1 g/L and 2.9 g/L higher at 2 and 4 years, respectively, for infants whose mothers received supplementation compared with placebo.[47] In our study at 24 weeks (approximately 6 months), maternal LNS, with and without infant ARV therapy (mLNS and mLNS-iARV), was associated with approximately 4–5 g/L lower infant Hb at 24 weeks, which contradicts the 6-month findings in the Tanzanian sample.[47] These differences may be due to varying time periods of supplementation (LNS in our sample was initiated postpartum) or our ability to account for the effects of inflammation/infection on Hb values.

There are several limitations to this work. Initial subsample measurements were obtained at 2- or 6-week postpartum due to insufficient plasma at 2 weeks. This reflects varying periods of exposure to the interventions; therefore, we evaluated for intervention effects separately at each visit in the subsample analyses. Compared with all randomized infants, included infants were healthier and worse off infants (including those with HIV infection) and mothers were lost to follow-up or excluded from analyses. As such, our ability to detect intervention effects may be reduced. Some mothers received iron supplementation when clinically indicated; however, sensitivity analyses showed that this did not change our interpretation of intervention effects. Finally, our findings may not be generalizable to other populations of HIV-infected women and their HIV-exposed infants, as mothers with low CD4 counts and previous ARV exposure were excluded from BAN and also due to our selection criteria for longitudinal analysis. Dyads were closely followed by study staff and promptly treated for adverse events and other illnesses.

The WHO now recommends that HIV-infected mothers or their uninfected infants receive ARV prophylaxis during 12 months of breastfeeding.[1] Given these revised recommendations, the nutritional demands of lactation and HIV,[48] and the adoption of lifelong ARV therapy for pregnant and lactating HIV-infected women (option B+), maternal LNS or possibly another iron supplementation method (eg, iron–folic acid) may be valuable to support lactation and prevent impaired iron status and associated consequences such as elevated risk of maternal or infant mortality and maternal HIV-disease progression.[5,7,8] However, in settings with endemic malaria, iron supplementation may be associated with increased risk of malaria[49,50]; thus, supplementation should be coupled with malaria prevention, diagnosis, and treatment efforts. Future randomized controlled trials are needed to examine the effects of ARV regimens beyond 6 months and to establish the optimal composition of supplementation to minimize possible adverse effects of extended ARV regimens. If adverse effects of extended regimens on iron status are observed in clinical trials, it may be possible to provide supplementation along with ARV therapy to support maternal health and micronutrient status through option B+ programming. Further evidence, however, is needed to establish the feasibility and acceptability of providing maternal supplementation in conjunction with ARV therapy and malaria diagnosis, prevention, and treatment efforts.

ACKNOWLEDGMENTS

The authors thank all the women and infants who participated in the study.

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APPENDIX 1

We like to thank the members of the BAN study team: Linda Adair, Yusuf Ahmed, Mounir Ait-Khaled, Sandra Albrecht, Shrikant Bangdiwala, Ronald Bayer, Margaret Bentley, Brian Bramson, Emily Bobrow, Nicola Boyle, Sal Butera, Charles Chasela, Charity Chavula, Joseph Chimerang'ambe, Maggie Chigwenembe, Maria Chikasema, Norah Chikhungu, David Chilongozi, Grace Chiudzu, Lenesi Chome, Anne Cole, Amanda Corbett, Amy Corneli, Anna Dow, Ann Duerr, Henry Eliya, Sascha Ellington, Joseph Eron, Sherry Farr, Yvonne Owens Ferguson, Susan Fiscus, Valerie Flax, Ali Fokar, Shannon Galvin, Laura Guay, Chad Heilig, Irving Hoffman, Elizabeth Hooten, Mina Hosseinipour, Michael Hudgens, Stacy Hurst, Lisa Hyde, Denise Jamieson, George Joaki (deceased), David Jones, Elizabeth Jordan-Bell, Zebrone Kacheche, Esmie Kamanga, Gift Kamanga, Coxcilly Kampani, Portia Kamthunzi, Deborah Kamwendo, Cecilia Kanyama, Angela Kashuba, Damson Kathyola, Dumbani Kayira, Peter Kazembe, Caroline C. King, Rodney Knight, Athena P. Kourtis, Robert Krysiak, Jacob Kumwenda, Hana Lee, Edde Loeliger, Dustin Long, Misheck Luhanga, Victor Madhlopa, Maganizo Majawa, Alice Maida, Cheryl Marcus, Francis Martinson, Navdeep Thoofer, Chrissie Matiki (deceased), Douglas Mayers, Isabel Mayuni, Marita McDonough, Joyce Meme, Ceppie Merry, Khama Mita, Chimwemwe Mkomawanthu, Gertrude Mndala, Ibrahim Mndala, Agnes Moses, Albans Msika, Wezi Msungama, Beatrice Mtimuni, Jane Muita, Noel Mumba, Bonface Musis, Charles Mwansambo, Gerald Mwapasa, Jacqueline Nkhoma, Megan Parker, Richard Pendame, Ellen Piwoz, Byron Raines, Zane Ramdas, John Rublein, Mairin Ryan, Ian Sanne, Christopher Sellers, Diane Shugars, Dorothy Sichali, Wendy Snowden, Alice Soko, Allison Spensley, Jean-Marc Steens, Gerald Tegha, Martin Tembo, Roshan Thomas, Hsiao-Chuan Tien, Beth Tohill, Charles van der Horst, Esther Waalberg, Elizabeth Widen, Jeffrey Wiener, Cathy Wilfert, Patricia Wiyo, Innocent Zgambo, and Chifundo Zimba.

The BAN study was supported by grants from the Prevention Research Centers Special Interest Project of the Centers for Disease Control and Prevention (SIP 13-01 U48-CCU409660-09, SIP 26-04 U48-DP000059-01, and SIP 22-09 U48-DP001944-01); the National Institute of Allergy and Infectious Diseases; the University of North Carolina Center for AIDS Research (P30-AI50410); and the NIH Fogarty AIDS International Training and Research Program (DHHS/NIH/FIC 2-D43 TW01039-06 and R24 TW007988; the American Recovery and Reinvestment Act). The Malawi Mothers and Infants (MaMi) study was supported by a grant from the Bill and Melinda Gates Foundation (OPP53107) and the Carolina Population Center (R24 HD050924). The antiretrovirals used were donated by Abbott Laboratories, GlaxoSmithKline, Boehringer Ingelheim, Roche Pharmaceuticals, and Bristol-Myers Squibb. The Call to Action for the Preventing Mother-to-Child Transmission programme was supported by the Elizabeth Glaser Pediatric AIDS Foundation, the United Nations Children's Fund, the World Food Program, the Malawi Ministry of Health and Population, Johnson and Johnson, and the US Agency for International Development.