The Journal of Nutrition
Community and International Nutrition

Plasma Micronutrient Concentrations Are Altered by Antiretroviral Therapy and Lipid-Based Nutrient Supplements in Lactating HIV-Infected Malawian Women[1–3]

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Abstract

Background: Little is known about the influence of antiretroviral therapy with or without micronutrient supplementation on the micronutrient concentrations of HIV-infected lactating women in resource-constrained settings.

Objective: We examined associations of highly active antiretroviral therapy (HAART) and lipid-based nutrient supplements (LNS) with concentrations of selected micronutrients in HIV-infected Malawian women at 24 wk postpartum.

Methods: Plasma micronutrient concentrations were measured in a subsample ($n = 690$) of Breastfeeding, Antiretrovirals, and Nutrition (BAN) study participants who were randomly assigned at delivery to receive HAART, LNS, HAART+LNS, or no HAART/no LNS (control). HAART consisted of protease inhibitor–based triple therapy. LNS (140 g/d) met energy and micronutrient requirements of lactation. Multivariable linear regression tested the association of HAART and LNS, plus their interaction, with micronutrient concentrations, controlling for season, baseline viral load, and baseline CD4 count.

Results: We found significant HAART by LNS interactions for folate ($P = 0.051$), vitamin B-12 ($P < 0.001$), and transferrin receptors (TfRs) ($P = 0.085$). HAART was associated with lower folate (with LNS: −27%, $P < 0.001$; without LNS: −12%, $P = 0.040$) and higher TfR concentrations (with LNS: +14%, $P = 0.004$; without LNS: +28%, $P < 0.001$), indicating iron deficiency. LNS increased folate (with HAART: +17%, $P = 0.037$; without HAART: +39%, $P < 0.001$) and decreased TfR concentrations (with HAART only: −12%, $P = 0.023$). HAART was associated with lower vitamin B-12 concentrations only when LNS was present (−18%, $P = 0.001$), whereas LNS increased vitamin B-12 only when no HAART was present (+27%, $P < 0.001$). HAART, but not LNS, was associated with higher retinol-binding protein (RBP; +10%, $P = 0.007$). We detected no association of HAART or LNS with selenium, ferritin, or hemoglobin.

Conclusion: The association of HAART with lower folate, iron deficiency, and higher RBP plus the attenuation of LNS effects on folate and vitamin B-12 when combined with HAART has implications for the health of lactating HIV-infected women taking HAART in prevention of mother-to-child transmission programs. This trial was registered at clinicaltrials.gov as NCT00164736. *J Nutr* 2015;145:1950–7.

Keywords: highly active antiretroviral therapy, lipid-based nutrient supplements, micronutrient, HIV, mothers

Introduction

The WHO's latest update on the use of antiretrovirals for the prevention of mother-to-child transmission (PMTCT)[12] has precipitated a shift toward the provision of antiretrovirals to mothers rather than to infants in African countries with high HIV prevalence (1, 2). More than 1.5 million women are now

[1] The Breastfeeding, Antiretrovirals, and Nutrition (BAN) study was supported by grants from the Prevention Research Center's Special Interest Project of the CDC (SIP 13-01 U48-CCU409660-09, SIP 26-04 U48-DP000059-01, and SIP 22-09 U48-DP001944-01), the Bill & Melinda Gates Foundation (OPP53107), the National Institute of Allergy and Infectious Diseases, the University of North Carolina Center for AIDS Research (P30-AI50410), the Carolina Population Center (R24 HD050924), the NIH Fogarty AIDS International Training and Research Program (DHHS/NIH/FIC 2-D43 TW001039), and Fogarty International Clinical Research Scholars (R24TW007988; the American Recovery and Reinvestment Act). The antiretrovirals used in the BAN study were donated by Abbott Laboratories, GlaxoSmithKline, Boehringer Ingelheim, Roche Pharmaceuticals,

Manuscript received February 11, 2015. Initial review completed March 15, 2015. Revision accepted June 5, 2015.
First published online July 8, 2015; doi:10.3945/jn.115.212290.

eligible to receive highly active antiretroviral therapy (HAART), containing a combination of 3 drugs, either during pregnancy and breastfeeding (Option B) or starting in pregnancy and continuing for life (Option B+) (3). Although these strategies have clear benefits for preventing vertical transmission, the long-term effects of HAART on the micronutrient status of women participating in PMTCT programs are not well characterized.

Marginal micronutrient status and deficiencies are common among HIV-infected women in resource-limited countries (4–7). In patients with low CD4 counts, HAART has a beneficial impact on micronutrient status through reductions in anemia (8–10) and vitamin A (11) and selenium deficiency (12). It is not known if these positive effects of HAART on the status of some micronutrients occur in individuals with higher CD4 and/or minimal HIV disease progression, like many mothers in PMTCT programs. Some negative effects of antiretrovirals on micronutrient concentrations have been reported, particularly for vitamins D (13, 14) and B-12 (15, 16). Negative associations of antiretrovirals used for PMTCT with maternal micronutrient concentrations have implications for the health of the mother and her child, through micronutrient stores obtained in utero and micronutrients available in breast milk. In the pre-HAART era, micronutrient supplementation provided to people living with HIV improved their hematologic status (17–25). However, the association of micronutrient supplementation with plasma micronutrient concentrations in lactating women receiving HAART is unknown.

This article presents data from a selected subsample of women who participated in the Breastfeeding, Antiretrovirals, and Nutrition (BAN) study in Malawi. We examine the association of micronutrient-fortified lipid-based nutrient supplements (LNS) and protease inhibitor–based HAART regimens on maternal micronutrient concentrations. We focus on associations at 24 wk, the time at which participants had their longest exposure to study interventions.

Methods

Subjects and procedures. From 2004 to 2009, HIV-1–infected, pregnant women were recruited into the BAN study at antenatal clinics in Lilongwe, Malawi. They received the standard of care during pregnancy from local health facilities. Mother-infant pairs were eligible for enrollment at delivery if infants had a birth weight ≥2 kg and mothers had a CD4 count ≥250 cells/mm^3 (≥200 cells/mm^3 until July 2006), hemoglobin ≥ 70 g/L, and no previous antiretroviral use (26).

At delivery, women were randomly assigned to the following maternal interventions: HAART, LNS, HAART+LNS, and no HAART/no LNS (control). Micronutrient biomarkers were analyzed in a subsample of stored plasma from BAN participants. We used all 24-wk specimens with a matched infant sample and available anthropometric and dietary data. Mothers were excluded if they had multiple births or their infants became HIV-positive, because we also planned to use the subsample to analyze the relation between maternal supplementation and infant growth. Multiples and HIV-positive infants have different rates of growth than do singletons and HIV-negative infants.

Nutrition and antiretroviral interventions were provided from delivery through 28 wk postpartum. Mothers were counseled to exclusively breastfeed from 0 to 24 wk and to wean their infants between 24 and 28 wk. LNS (Nutriset), containing peanut paste, nonfat milk powder, sugar, vegetable oil, and micronutrient mix, was used for the nutrition intervention. The daily LNS dose (140 g) was designed to supply the additional energy, protein, and micronutrient needs of lactation. It provided 3120 kJ (746 kcal) of energy, 20.8 g protein, and 16 vitamins and minerals. The complete nutrient content of the LNS is described elsewhere (27). Briefly, it contained 300 μg folic acid (0.6 times the RDA for lactating women), 2.6 μg cyanocobalamin (0.9 times the RDA), 75 μg sodium selenite (1.3 times the RDA), and 15 mg ferrous sulfate (1.7 times the RDA) (28–30). The LNS did not contain vitamin A due to evidence available before the start of the study that it could increase HIV transmission through breast milk (31).

The maternal antiretroviral intervention was a HAART regimen containing 3 drugs (32). All women assigned to the antiretroviral arms received lamivudine/zidovudine as a single pill (Combivir; GlaxoSmithKline) throughout the intervention period (0–28 wk). In addition, the first 39 BAN participants randomly assigned to antiretrovirals received nevirapine as their study drug. The study switched to the second-line drug, nelfinavir (Viracept; Roche), which was given to the next 146 women, after the FDA issued a black box warning concerning the use of nevirapine in women with a CD4 count >250 cells/mm^3. A further change was made to lopinavir/ritonavir (LPVr; Kaletra; Abbott) for reasons of availability, safety, and potency. Nelfinavir and LPVr are protease inhibitors, which have side effects including nausea, diarrhea, increased lipids, and lipodystrophy (33). In our micronutrient subsample, 3 women switched from nevirapine to nelfinavir, one switched from nelfinavir to LPVr, and one switched from LPVr to nelfinavir (due to reactions to LPVr). We coded these women as taking the drug used at the 24-wk visit.

Venous blood samples were collected at 24 wk postpartum. Plasma was separated from RBCs within 60 min, separated into aliquots in polypropylene storage tubes, and kept at −70°C. Mothers were asked to report their adherence to the LNS at 1, 4, 8, 12, and 21 wk. Adherence to HAART was based on pill counts at 4, 12, and 18 wk. This was calculated by using the following formula: (number of pills distributed at previous visit – number of pills returned at current visit)/(days between visits × pills prescribed per day). Adherence to the LNS regimen was obtained by questionnaire during regular study visits. Mothers were asked how much of the supplement they ate yesterday in half-packet increments, ranging from none to 2 packets (the full daily dose). A questionnaire on socioeconomic characteristics was administered to mothers during screening.

At the time when this study began, there was not yet evidence that antiretrovirals were effective at preventing HIV transmission through breast milk and it was acceptable to have study groups without drugs. In 2008, the study's data safety and monitoring board stopped enrollment in the control arm when it became clear that use of antiretrovirals prevented transmission. Ethical approval for the study was obtained from the Malawi National Health Science Research Committee and the institutional review boards at the University of North Carolina at Chapel Hill, the US CDC, and the University of California, Davis. The trial was registered at clinicaltrials.gov (NCT00164736).

Laboratory analysis. Plasma concentrations of most micronutrient biomarkers were measured at the USDA–Agricultural Research Service Western Human Nutrition Research Center. Vitamin B-12 and folate were analyzed by using the SimulTRAC-SNB Radioassay Kit [vitamin B-12 (57Co)/folate (12585 I); MP Biomedicals]. The analysis of retinol-binding protein (RBP) was performed with the Human Retinol BP ELISA, an immunoperoxidase assay for the determination of RBP in

and Bristol-Myers Squibb. The Call to Action Prevention of Mother-to-Child Transmission Program, from which BAN mothers were recruited, 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 & Johnson, and the US Agency for International Development.

[2] Author disclosures: VL Flax, LS Adair, LH Allen, S Shahab-Ferdows, D Hampel, CS Chasela, G Tegha, EJ Daza, A Corbett, NL Davis, D Kamwendo, AP Kourtis, DJ Jamieson, and ME Bentley, no conflicts of interest. CM van der Horst received grant support from Abbott Laboratories and GlaxoSmithKline.

[3] Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

[12] Abbreviations used: AGP, α-1-acid glycoprotein; BAN, Breastfeeding, Antiretrovirals, and Nutrition; CRP, C-reactive protein; HAART, highly active antiretroviral therapy; LNS, lipid-based nutrient supplements; LPVr, lopinavir/ritonavir; MRP, multidrug resistance–related protein; PMTCT, prevention of mother-to-child transmission; RBP, retinol-binding protein; TfR, transferrin receptor.

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TABLE 1 Characteristics of mothers in the micronutrient analysis subsample of the BAN study[1]

	No LNS/no HAART (n = 237)	LNS (n = 238)	HAART (n = 104)	LNS+HAART (n = 111)	P
Age, y	26.6 ± 5.0	26.6 ± 5.1	27.3 ± 4.9	26.0 ± 5.2	0.36
More than primary education, %	34	39	37	35	0.64
Number of pregnancies	3.4 ± 1.7	3.3 ± 1.6	3.4 ± 1.4	3.4 ± 1.8	0.96
BMI					
At 24 wk, kg/m^2	22.7 ± 2.8	22.8 ± 3.4	22.3 ± 3.3	22.3 ± 2.6	0.29
<18.5 kg/m^2 at 24 wk, %	4	4	7	6	0.62
Viral load at baseline,[2] $\log_{10}$ copies	4.1 ± 0.9	4.1 ± 0.9	4.2 ± 0.9	4.2 ± 0.9	0.93
CD4					
At baseline,[2] cells/μL	428 (327, 577)	444 (323, 600)	460 (304, 571)	449 (336, 580)	0.90
At 24 wk,[3] cells/μL	474 (326, 655)	462 (337, 698)	634 (470, 774)	628 (434, 796)	<0.001
<250 cells/mm^3 at 24 wk, %	9	10	2	4	0.03
Hemoglobin at baseline,[2] g/L	109 ± 1.2	107 ± 1.2	107 ± 1.2	108 ± 1.1	0.33
Anemia					
Hemoglobin <120 g/L at baseline,[2] %	54	58	55	52	0.76
Hemoglobin <120 g/L at 24 wk, %	36	32	38	35	0.75
CRP >5 mg/L at 24 wk, %	19	16	18	14	0.69
AGP >1 g/L at 24 wk, %	36	35	34	31	0.82

[1] Values are means ± SDs or medians (IQRs) unless otherwise indicated; n = 690. AGP, α-1-acid glycoprotein; BAN, Breastfeeding, Antiretrovirals, and Nutrition; CRP, C-reactive protein; HAART, highly active antiretroviral therapy; LNS, lipid-based nutrient supplements.
[2] Baseline viral load, CD4, hemoglobin, and anemia were measured during pregnancy when participants were screened.
[3] CD4 at 24 wk: no LNS/no HAART, n = 211; LNS, n = 213; HAART, n = 92; LNS + HAART, n = 105.

human sera (Immunology Consultants Laboratory). Transferrin receptors (TfRs) and inflammatory markers [C-reactive protein (CRP) and α-1-acid glycoprotein (AGP)] were measured by using a Cobas Integra 400+ analyzer (Roche Diagnostics). Ferritin concentrations were determined with the IRMA Ferritin Coat-a-Count radioimmunoassay (Siemens Health Care Diagnostics).

Selenium concentrations were analyzed at the USDA–Agricultural Research Service Grand Forks Human Nutrition Research Center by automated electrothermal atomic absorption spectrophotometry. Hemoglobin was measured in whole blood in Lilongwe by using a Beckman Coulter AcT 5-part Differential Analyzer (Beckman Coulter).

Statistical analysis. Differences in background characteristics of participants and adherence to LNS and HAART by study group were examined by using ANOVA for continuous variables and chi-square tests for categorical variables. Natural-log transformations were used for all micronutrient outcome variables because they followed non-Gaussian distributions; outcomes were modeled as continuous variables. Multivariable linear regression was used to test associations between the LNS and HAART interventions and maternal plasma micronutrient concentrations. Adjusted geometric means for each intervention group and ratios for pairs of interventions (e.g., HAART vs. no HAART) and their 95% CIs were calculated from the models. A HAART × LNS interaction term was included in all initial models and retained if $P < 0.10$. For micronutrients with significant HAART × LNS interactions, exploratory analyses were conducted to examine possible differential effects of regimens containing LPVr + Combivir or nelfinavir + Combivir. In exploratory models, we estimated ratios of geometric means for pairs of groups (e.g., LPVr vs. no HAART among women receiving LNS). All models controlled for baseline CD4 count and $\log_{10}$ viral load as

TABLE 2 Folate, vitamin B-12, and TfR concentrations at 24 wk postpartum in a sample of BAN study mothers who were untreated or given HAART with or without LNS[1]

	HAART	No HAART	Ratio of HAART to no HAART (95% CI)
Folate, nmol/L			
LNS	19.1 (17.1, 21.1)	26.0 (24.2, 27.8)	0.73*** (0.65, 0.83)
No LNS	16.3 (14.6, 18.0)	18.7 (17.4, 20.0)	0.88* (0.77, 0.99)
Ratio of LNS to no LNS	1.17* (1.01, 1.35)	1.39*** (1.26, 1.54)	
Vitamin B-12, pmol/L			
LNS	286 (257.4, 313.7)	349 (325.7, 372.2)	0.82** (0.73, 0.92)
No LNS	310 (278.4, 341.1)	276 (257.4, 294.1)	1.12 (0.99, 1.27)
Ratio of LNS to no LNS	0.92 (0.80, 1.06)	1.27*** (1.15, 1.39)	
TfR, mg/L			
LNS	5.0 (4.6, 5.3)	4.3 (4.1, 4.6)	1.14** (1.04, 1.25)
No LNS	5.6 (5.2, 6.0)	4.4 (4.2, 4.6)	1.28*** (1.17, 1.40)
Ratio of LNS to no LNS	0.88* (0.79, 0.98)	0.98 (0.92, 1.06)	

[1] Values are adjusted geometric means (95% CIs) or ratios (95% CIs). Folate and vitamin B-12: no HAART, no LNS, n = 237; no HAART, LNS, n = 238; HAART, no LNS, n = 103; HAART, LNS, n = 110. TfR: no HAART, no LNS, n = 237; no HAART, LNS, n = 238; HAART, no LNS, n = 104; HAART, LNS, n = 111. Models controlled for season, baseline CD4 count, baseline viral load, and use of folate inhibitors (for folate only) and included significant HAART × LNS interactions (folate: P = 0.051; vitamin B-12: $P < 0.001$; TfR: P = 0.085). *,**,***Significant ratio: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. BAN, Breastfeeding, Antiretrovirals, and Nutrition; HAART, highly active antiretroviral therapy; LNS, lipid-based nutrient supplements; TfR, transferrin receptor.

continuous variables. Season at the time of the 24-wk visit was also included in the models to control for potential differences in dietary intake and to account for the possibility that calendar time was related to the outcomes. Season was included as a binary variable denoting the presence or absence of the food-insecure period of the year (during the rainy season) based on the month and date of the woman's study visit. Approximately 10% of the analysis sample received either sulfadoxine-pyrimethimine or cotrimoxazole (drugs with folate-inhibiting properties) during the 3 wk preceding the study visit when blood was collected. Consequently, the presence or absence of folate-inhibiting drugs was included in the folate model. To better understand the role of inflammation on the association of antiretrovirals with micronutrients, we compared multivariable linear regression models with and without markers of inflammation (measured as log CRP and log AGP and modeled as continuous variables) for biomarkers that are known to be influenced by the acute phase response (selenium, RBP, ferritin, TfR, and hemoglobin) (34).

Results

Of 709 women selected for the micronutrient subsample at 24 wk, 18 were dropped from the analysis. Nine of these stopped taking their drugs before 24 wk and 9 were taking nevirapine, a sample that was too small to produce stable estimates in regression models. There were no significant differences by study group in age, level of education, number of pregnancies, BMI, baseline viral load or CD4 count, anemia, high CRP, or high AGP (**Table 1**). As expected, we found significantly lower median CD4 counts and percentage of CD4 <250 cells/mm^3 among women in the groups that received no HAART at 24 wk compared with those who received HAART. Characteristics of mothers in the micronutrient subsample compared with those of other BAN participants are shown in **Supplemental Table 1**.

Adherence to LNS and HAART was high and generally increased over time. The percentages of mothers who reported consuming the full dose (2 packets) of LNS the previous day were as follows: 1 wk, 87%; 4 wk, 89%; 8 wk, 94%; 12 wk, 94%; and 21 wk, 96%. On the basis of pill counts, mean drug adherence was 86% at 4 wk, 87% at 12 wk, and 90% at 18 wk. LNS adherence did not differ significantly between the LNS and HAART+LNS groups at any visit. Similarly, there were no differences in drug adherence by type of HAART or between the groups receiving HAART with or without LNS.

We found significant interactions of HAART and LNS for folate ($P = 0.051$), vitamin B-12 ($P < 0.001$), and TfR ($P = 0.085$) but not for selenium, RBP, ferritin, or hemoglobin. Folate concentrations were higher among women receiving LNS with HAART (+17%; $P = 0.037$) or without HAART (+39%; $P < 0.001$) (**Table 2**). HAART was associated with lower folate concentrations in women receiving LNS (−27%; $P < 0.001$) or no LNS (−12%; $P = 0.040$). Vitamin B-12 concentrations were higher among women who received LNS and no HAART than in those receiving no LNS and no HAART (+27%; $P < 0.001$). HAART was associated with lower vitamin B-12 concentrations in women who received LNS (−18%; $P = 0.001$) but not in those with no LNS. Compared with women not receiving HAART, TfR concentrations were higher in women receiving HAART with (+14%; $P = 0.004$) or without (+28%; $P < 0.001$) LNS. Among women receiving HAART, TfR concentrations were lower in women receiving LNS (−12%; $P = 0.023$).

In exploratory analyses by type of drug regimen, the ratio of the geometric means indicated that women taking LPVr had lower folate concentrations if they received LNS (−35%; $P < 0.001$) or no LNS (−17%; $P = 0.031$), whereas women taking nelfinavir had lower folate only if they received LNS (−25%; $P = 0.006$)

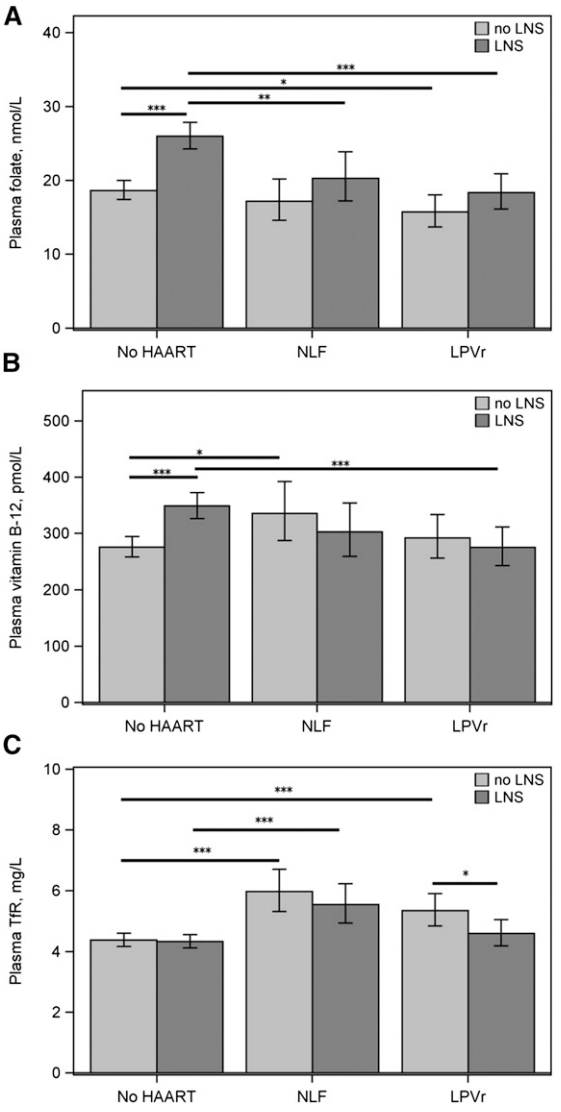

FIGURE 1 Adjusted geometric mean plasma folate (A), vitamin B-12 (B), and TfR (C) concentrations at 24 wk postpartum in a sample of BAN study mothers who were untreated or given HAART with or without LNS. Values are geometric means and 95% CIs. (A and B) No HAART, no LNS: $n = 237$; no HAART, LNS: $n = 238$; NLF, no LNS: $n = 43$; NLF, LNS: $n = 43$; LPVr, no LNS: $n = 60$; LPVr, LNS: $n = 67$. (C) No HAART, no LNS: $n = 237$; no HAART, LNS: $n = 238$; NLF, no LNS: $n = 44$; NLF, LNS: $n = 44$; LPVr, no LNS: $n = 60$; LPVr, LNS: $n = 67$. Comparisons were made between no LNS and LNS within each drug category (no HAART, NLF, and LPVr) and between NLF or LPVr and no HAART within each LNS category. *,**,***Difference between adjusted means at either end of the bar: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. BAN, Breastfeeding, Antiretrovirals, and Nutrition; HAART, highly active antiretroviral therapy; LNS, lipid-based nutrient supplements; LPVr, lopinavir/ritonavir; NLF, nelfinavir; TfR, transferrin receptor.

(**Figure 1**A). Women taking LPVr had lower vitamin B-12 concentrations if they received LNS (−24%; $P = 0.001$) but not if they did not receive LNS (Figure 1B). In contrast, women taking nelfinavir had higher vitamin B-12 concentrations if they received no LNS (+20%; $P < 0.022$) but not in those who received LNS. LNS increased folate and vitamin B-12 concentrations in women receiving no drugs (folate: +33%, $P < 0.001$; vitamin B-12: +24%, $P < 0.001$) but not in women receiving either LPVr or nelfinavir. Women taking LPVr had higher TfR

TABLE 3 Selenium, RBP, ferritin, and hemoglobin concentrations at 24 wk postpartum in a sample of BAN study mothers who were untreated or given HAART with or without LNS[1]

	HAART	No HAART	Ratio of HAART to no HAART	LNS	No LNS	Ratio of LNS to no LNS
Selenium, μg/L	82.0 (79.5, 84.6)	81.7 (80.0, 83.4)	1.00 (0.97, 1.04)	82.9 (80.8, 84.9)	80.7 (78.8, 82.7)	1.03 (0.99, 1.06)
RBP, μmol/L	0.98 (0.92, 1.03)	0.89 (0.86, 0.93)	1.10** (1.03, 1.17)	0.92 (0.88, 0.96)	0.92 (0.88, 0.96)	1.00 (0.94, 1.07)
Ferritin, μg/L	22.4 (19.8, 24.9)	24.5 (22.6, 26.4)	0.91 (0.79, 1.05)	24.7 (22.5, 27.0)	23.0 (20.9, 25.0)	1.08 (0.95, 1.22)
Hemoglobin, g/L	123 (121.8, 125.1)	123 (121.5, 123.7)	1.00 (0.99, 1.02)	123 (121.8, 124.4)	123 (121.2, 123.9)	1.00 (0.99, 1.02)

[1] Values are adjusted geometric means (95% CIs) or ratios (95% CIs). Selenium and hemoglobin: HAART, $n = 214$; no HAART, $n = 474$; LNS, $n = 348$; no LNS, $n = 340$. RBP: HAART, $n = 214$; no HAART, $n = 473$; LNS, $n = 348$; no LNS, $n = 339$. Ferritin: HAART, $n = 215$; no HAART, $n = 475$; LNS, $n = 349$; no LNS, $n = 341$. Models controlled for season, baseline CD4 count, and baseline viral load. HAART × LNS interactions were not significant (selenium: $P = 0.45$; RBP: $P = 0.39$; ferritin: $P = 0.55$; hemoglobin: $P = 0.66$). **Significant ratio, $P < 0.01$. BAN, Breastfeeding, Antiretrovirals, and Nutrition; HAART, highly active antiretroviral therapy; LNS, lipid-based nutrient supplements; RBP, retinol-binding protein.

only if they received no LNS (+20%; $P < 0.001$) (Figure 1C). Women taking nelfinavir had higher TfR if they received LNS (+25%; $P < 0.001$) or no LNS (+31%; $P < 0.001$). LNS was associated with lower TfR only in women receiving LPVr (−15%; $P = 0.031$); a similar association was not detected among women receiving nelfinavir.

We found no significant interactions of HAART and LNS for RBP, selenium, ferritin, or hemoglobin (**Table 3**). RBP concentrations were higher among women taking HAART than among those with no HAART (RBP: +10%; $P = 0.007$), but no associations with LNS were detected. There were no associations of either intervention with concentrations of selenium, ferritin, or hemoglobin.

CRP, an acute phase protein, was negatively associated with RBP ($P < 0.001$) and hemoglobin ($P < 0.001$) and positively associated with ferritin ($P < 0.001$). AGP, a marker of chronic infection, was negatively associated with selenium ($P < 0.001$) and hemoglobin ($P < 0.001$). Including CRP and AGP in the models did not appreciably change the coefficients for selenium, RBP, ferritin, TfR, or hemoglobin (data not shown).

Discussion

This study examined the association of protease inhibitor–based HAART and LNS with maternal micronutrient concentrations after 24 wk of use. Women in this subsample had high mean CD4 counts and were assigned to HAART to test whether it prevented HIV transmission to their infants. Micronutrient concentrations and supplementation interventions have not been studied previously in women taking HAART for PMTCT. Yet, such women represent an important and growing population of HAART users due to the recent changes in recommendations to provide lifelong antiretrovirals to mothers in Option B+ PMTCT programs and to initiate HAART for all HIV-infected individuals with a CD4 count <500 cells/mm^3 (1, 2).

We found that maternal folate concentrations were higher in women receiving LNS and lower in women receiving HAART. Furthermore, HAART modified the effect of LNS on folate. Intermediate folate values for the combined interventions suggest that HAART diminished the benefits of supplementation, whereas a daily dose of 0.6 times the RDA of folic acid mitigated the negative influence of the drugs. Our finding that protease inhibitor–based therapy was associated with lower folate concentrations confirms results from small cross-sectional studies in children and adults (35, 36). Lower folate concentrations among participants receiving HAART could be related to poor absorption due to drug-related changes in gut epithelial integrity (37) or drug-related diarrhea, which commonly occurs with initiation of protease inhibitors and some other antiretroviral drugs (38, 39). It is possible that HAART is related to intracellular folate metabolism. Some classes of antiretrovirals inhibit multidrug resistance–related proteins (MRPs), including MRP3, which is involved in folic acid transport out of the gut (40). Folate and homocysteine have an inverse relation in HIV-infected individuals. Several studies found an association of antiretroviral therapy or duration of therapy with hyperhomocysteinemia and low folate concentrations (35, 36, 41, 42), whereas others detected no association (43–45). Additional research is needed to confirm the relation between folate and different types and combinations of antiretrovirals and to clarify the biological mechanisms.

In our sample, LNS supplementation was associated with higher vitamin B-12 concentrations only in women not taking HAART and HAART was associated with lower vitamin B-12 concentrations only in women receiving LNS, indicating that HAART eliminated the association of LNS with vitamin B-12. This was driven by the negative association of LPVr with vitamin B-12 in women receiving LNS. Other studies also showed that vitamin B-12 concentrations can be increased through vitamin B-12 supplementation in HIV-infected children and adults, especially if they are initially deficient (16, 46, 47). Evidence from previous research on the effects of antiretrovirals on vitamin B-12 concentrations or status is mixed. One study found that zidovudine treatment was associated with lower plasma vitamin B-12 concentrations (15). A second study showed that vitamin B-12 intake was related to larger increases in serum vitamin B-12 concentrations in individuals not taking protease inhibitors than in those taking protease inhibitors (16). However, a third study found that patients receiving HAART (type not described) had higher vitamin B-12 concentrations and were less likely to be vitamin B-12 deficient than a historical cohort of patients who were not taking HAART (48). The mixed evidence for the effects of antiretrovirals on vitamin B-12 concentrations indicates a need for further research in larger samples and with adequate information on the type of drugs and adherence.

The literature on the association of HAART and vitamin A concentrations is also mixed. Participants in this study had higher RBP concentrations when they received HAART, even when controlling for concurrent markers of inflammation and baseline CD4 and viral load. Because RBP has a 1:1 relation with serum retinol (49), this finding suggests that protease inhibitor–based HAART could contribute to improved vitamin A status in populations similar to that in the BAN study, with high baseline

CD4 and prevalent vitamin A deficiency. Our results agree with studies that showed that protease inhibitor–based HAART was related to higher RBP or β-carotene compared with individuals not receiving HAART (50, 51), whereas other studies found no differences in vitamin A concentrations by HAART status or type of HAART (12, 52). The variability in the literature points to the need to examine how baseline vitamin A status, initial CD4, and levels of inflammation influence changes in vitamin A concentrations before and after HAART initiation.

LNS alone was not associated with ferritin, TfR, or hemoglobin in this study. HAART was not associated with ferritin or hemoglobin but was associated with higher TfR concentrations, which indicates greater functional tissue iron deficiency in women taking these drugs. TfR can be elevated when there is iron-deficient erythropoiesis or tissue iron deficiency without anemia (53), which may be more common in people with chronic diseases and inflammation (54). The results on ferritin, TfR, and hemoglobin reported here confirm our earlier findings from a smaller, matched mother-infant subsample of BAN study participants (55).

Women in the BAN study received standard iron-folic acid tablets during pregnancy, as per Malawian guidelines, and their hemoglobin concentrations were ~120 g/L on average both after delivery and at 24 wk postpartum. This may explain the lack of LNS effect on hemoglobin in our sample, whereas increases in hemoglobin concentrations have been documented in HIV-infected pregnant and lactating women with low initial hemoglobin concentratons who were supplemented with multiple micronutrients (17). Our findings also differ from those of several studies that showed increases in hemoglobin concentrations in HIV patients after HAART initiation (8, 9, 56–58). Some possible reasons for these differences include the short duration of HAART therapy and inclusion of zidovudine in the HAART regimen in our study. HAART has a more pronounced effect on increasing hemoglobin in patients who have taken it for >6 mo (56), whereas the use of zidovudine-containing HAART causes lower hemoglobin concentrations in some individuals (56, 58, 59).

This study had 3 main limitations. First, participants were not randomly assigned to the different drugs. They took either nelfinavir+Combivir or LPVr+Combivir on the basis of the timing of their enrollment in the study. Although calendar time could be related to our outcomes, we tried to limit possible confounding by including season in the models. Second, this analysis used a purposively selected subsample of mothers enrolled in the BAN study. We initially planned to examine effects in women with or without LNS. This resulted in a subsample with relatively small numbers of participants in the drug groups, which could limit our ability to detect differences. Choosing a subsample can result in differences in characteristics from the randomized cohort. Women in the micronutrient subsample were slightly older and the proportion with low CD4 counts or anemia was larger than in the rest of the BAN participants. However, the actual differences between the subsample and other study participants for these indicators were so small as to be clinically insignificant. In addition, we controlled for baseline CD4 count and viral load to address the possibility of selection bias. Third, with the exception of hemoglobin, we did not measure baseline micronutrient concentrations. We made this choice because micronutrient values are typically lower in pregnancy, when screening occurred, and would not allow us to look at changes related to the interventions, which were implemented postpartum. Furthermore, we would not expect to find differences in participants' preintervention micronutrient concentrations in a randomized trial. We detected no differences between study arms in the proportion who were anemic at baseline, mean baseline hemoglobin, or any other baseline characteristic, suggesting that the groups were well balanced before initiating the study interventions.

The HAART regimens provided in this study were first-line treatment combinations at the time of study implementation and are now used as second-line regimens in PMTCT programs. On the basis of estimates of the proportion of patients who switch regimens due to treatment failure, the results of this study are applicable to ~100,000 women in sub-Saharan Africa who are receiving second-line drugs in PMTCT programs (3, 60). Anemia, iron deficiency, and inadequate folate status are common in HIV-infected pregnant and lactating women in Africa (61–63). If confirmed in larger studies, our findings on the association of HAART with folate and TfR concentrations have implications for the health of mothers and their infants and may require supplementation or other types of interventions. Given the recent rapid expansion of PMTCT programs, further research is urgently needed to quantify the effects of second-line HAART regimens and to study the association of first-line regimens with micronutrient concentrations of women in PMTCT.

Acknowledgments

We thank all of the members of the BAN Study Team at the University of North Carolina (UNC) Chapel Hill; the CDC, Atlanta; and the UNC Project in Lilongwe including: LSA, Yusuf Ahmed, Mounir Ait-Khaled, Sandra Albrecht, Shrikant Bangdiwala, Ronald Bayer, MEB, Brian Bramson, Emily Bobrow, Nicola Boyle, Sal Butera, CSC, Charity Chavula, Maggie Chigwenembe, Maria Chikasema, Norah Chikhungu, David Chilongozi, Joseph Chimerang'ambe, Grace Chiudzu, Lenesi Chome, Anne Cole, AC, Amy Corneli, Anna Dow, Ann Duerr, Henry Eliya, Sascha Ellington, Joseph Eron, Sherry Farr, Yvonne Owens Ferguson, Susan Fiscus, VLF, Ali Fokar, Shannon Galvin, Laura Guay, Chad Heilig, Irving Hoffman, Elizabeth Hooten, Mina Hosseinipour, Michael Hudgens, Stacy Hurst, Lisa Hyde, DJJ, George Joaki (deceased), David Jones, Elizabeth Jordan-Bell, Zebrone Kacheche, Esmie Kamanga, Gift Kamanga, Coxcilly Kampani, Portia Kamthunzi, DK, Cecilia Kanyama, Angela Kashuba, Damson Kathyola, Dumbani Kayira, Peter Kazembe, Caroline C King, Rodney Knight, APK, Robert Krysiak, Jacob Kumwenda, Hana Lee, Edde Loeliger, Dustin Long, Misheck Luhanga, Victor Madhlopa, Maganizo Majawa, Alice Maida, Cheryl Marcus, Francis Martinson, 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, GT, Martin Tembo, Roshan Thomas, Navdeep Thoofer, Hsiao-Chuan Tien, Beth Tohill, CMvdh, Esther Waalberg, Elizabeth Widen, Jeffrey Wiener, Cathy Wilfert, Patricia Wiyo, Innocent Zgambo, and Chifundo Zimba.

We also appreciate the selenium analyses carried out in Gerald Combs' laboratory by Laura Idso and Craig Lacher. LSA, CSC, GT, DK, APK, CMvdH, DJJ, and MEB designed and conducted the study; LHA, SS-F, and DH carried out the laboratory analyses; EJD was responsible for data management

and provided statistical advice; AC helped interpret findings; NLD provided data on antiretroviral adherence; and VLF carried out the statistical analysis, drafted the manuscript, and had primary responsibility for the final content. All authors read and approved the final manuscript.

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