Antiretroviral therapy provided to HIV-infected Malawian women in a randomized trial diminishes the positive effects of lipid-based nutrient supplements on breast-milk B vitamins[1–3]

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ABSTRACT

Background: Little information is available on B vitamin concentrations in human milk or on how they are affected by maternal B vitamin deficiencies, antiretroviral therapy, or maternal supplementation.

Objective: The objective was to evaluate the effects of antiretroviral therapy and/or lipid-based nutrient supplements (LNSs) on B vitamin concentrations in breast milk from HIV-infected women in Malawi.

Design: Breast milk was collected from 537 women recruited within the Breastfeeding, Antiretrovirals, and Nutrition study at 2 or 6 wk and 24 wk postpartum. Women were assigned to receive antiretrovirals and LNSs, antiretrovirals only, LNSs only, or a control. Antiretrovirals and LNSs were given to the mothers from weeks 0 to 28. The antiretrovirals were zidovudine/lamivudine and nelfinavir or lopinavir/ritonavir. LNSs provided 93–118% of the Recommended Dietary Allowances of thiamin, riboflavin, niacin, pyridoxine, and vitamin B-12. Infants were exclusively breastfed.

Results: LNSs increased milk concentrations of all vitamins except thiamin, whereas antiretrovirals lowered concentrations of nicotinamide, pyridoxal, and vitamin B-12. Although antiretrovirals alone had no significant effect on riboflavin concentrations, they negatively affected the LNS-induced increase in this vitamin. Thiamin was not influenced by the study interventions. Concentrations of all B vitamins were much lower than usually accepted values.

Conclusions: All B vitamins were low in milk, and all but thiamin were increased by maternal supplementation with LNSs. Antiretrovirals alone decreased concentrations of some B vitamins in milk. When LNS was given in addition to antiretrovirals, the negative effect of antiretrovirals offset the positive effect of LNSs for all vitamins except thiamin. This trial was registered at clinicaltrials.gov as NCT00164762. *Am J Clin Nutr* 2015;102:1468–74.

Keywords: antiretrovirals, B vitamins, breast milk, human milk, ultraperformance liquid chromatography tandem mass spectrometry

INTRODUCTION

The WHO recommends exclusive breastfeeding (EBF)[10] for the first 6 mo of life based on its positive benefits for infant health and development, and the assumption that it provides adequate amounts of all nutrients, including micronutrients (1). However, breastfeeding increases the risk of mother-to-child HIV transmission. Several studies have indicated that postnatal HIV-1 transmission from mother to child can be reduced by antiretroviral drugs given to the mother or infant (2–9). These findings led to the current WHO recommendations for HIV-infected mothers to breastfeed exclusively for 6 mo and to continue breastfeeding to 12 mo in conjunction with taking antiretrovirals (either the mother or infant) for the duration of breastfeeding (1).

HIV infection increases energy and nutrient requirements, which makes it more challenging to meet the needs of both the mother and the infant (10, 11). No information is available concerning whether antiretroviral therapy provided to HIV-infected mothers during lactation affects micronutrient concentrations in breast milk. This would be of greatest concern in areas where maternal micronutrient status is poor because inadequate intake or the status of many micronutrients, especially B vitamins, adversely affects the amounts secreted in milk (12). Lipid-based nutrient supplements (LNSs) could increase the supply of energy and micronutrients to the HIV-positive mother, and the secretion of some micronutrients in her milk; however, the efficacy of this approach has not been evaluated (13, 14).

This study examines the effects of antiretrovirals and LNSs on thiamin, riboflavin, nicotinamide, pyridoxal, and vitamin B-12 concentrations in the breast milk of HIV-infected Malawian mothers. Values for the concentration of each B vitamin were compared with those published for milk from well-nourished women and used to set Adequate Intakes (AIs) for infants aged 0–6 mo (15).

METHODS

Subjects and sample collection

The Breastfeeding, Antiretrovirals, and Nutrition study population consisted of HIV-infected women recruited from 4 antenatal clinics in Lilongwe, Malawi, between 2004 and 2009. The recruitment methods and flow of participants have been described in detail elsewhere (8, 11, 16, 17). Mothers in this study were counseled to exclusively breastfeed for 24 wk and then to rapidly wean from 24 to 28 wk, in accordance with WHO recommendations at the time the study began (16, 18).

At delivery, women and their infants were randomly assigned by using the permuted block method to a 2-arm (LNSs or no LNSs) by 3-arm (maternal antiretrovirals, infant antiretrovirals, or neither) factorial design (**Supplemental Figure 1**). For the current analysis of the effects on breast milk, no differentiation was made between infants treated or not treated with antiretrovirals, which resulted in 4 intervention groups. After screening and enrollment by study nurses, a data manager generated the random allocation sequence. Study pharmacists assigned participants to interventions via sequentially numbered envelopes and distributed antiretrovirals and LNSs during the scheduled visits at 0, 1, 2, 4, 6, 8, 12, 18, 21, 24, and 28 wk postpartum. The LNS was manufactured by Nutriset in France (www.nutriset.fr). Each sachet contained 70 g, and the women were instructed to consume 2 sachets/d, which provided 746 kcal/d and approximately the Recommended Dietary Allowance for most micronutrients (**Table 1**). However, the LNS provided no vitamin A because of concerns that supplements could increase HIV transmission through breast milk (19, 20). The antiretroviral drug regimen for mothers consisted of zidovudine and lamivudine (Combivir) plus either nevirapine (until January 2005), nelfinavir (from January 2005 to February 2006), or lopinavir and ritonavir (Kaletra, Abbott; from February 2006 until study completion). In addition, 2 kg maize flour was provided weekly to all participants for family consumption.

Maternal reports of LNS consumption were taken at 1, 4, 8, 12, and 21 wk. Breast milk was manually expressed by the participants, producing opportunistic samples during the regular study visits at

weeks 2, 6, and 24. The milk was immediately frozen after collection. The 6-wk samples were obtained when a 2-wk sample was unavailable or the infant had insufficient plasma at 2 wk. The sample size was predetermined by the number of participants providing milk, maternal, and infant plasma samples; participants were prioritized for selection in the micronutrient subsample if they had anthropometric and dietary intake data. They were excluded from the subsample if the infants became HIV-positive or were multiple births. The larger study's sample size calculations were previously described in detail (16). As with all food-based nutrition supplement trials, participants and physicians could not be blinded to the study arm. Outcome assessors and data analysts were not informed about the allocation strategy.

No milk samples were collected before supplementation with LNS; therefore, no true baseline concentrations were available for control in the statistical analyses. Samples were shipped on dry ice to the CDC in Atlanta and stored at $-80°C$ until analyzed at the USDA/Agricultural Research Service, Western Human Nutrition Research Center in Davis, CA.

This research was approved by the Malawi National Health Science Research Committee, the Institutional Review Boards at the University of North Carolina Chapel Hill, the CDC, and the University of California, Davis. The trial was monitored for safety and efficacy by the National Institute of Allergy and Infectious Diseases Vaccine and Prevention Data and Safety Monitoring Board.

Biochemical analyses

Free thiamin, riboflavin, flavin adenine dinucleotide (FAD), nicotinamide, and pyridoxal in milk were analyzed simultaneously by

TABLE 1
Composition of the lipid-based nutrient supplement formulated for use by lactating women aged 19–30 y[1]

Nutrient	RDA for lactating women	Amount per 2 packets (140 g)
Energy		
(kcal)	—	746
(kJ)	—	3120
Protein, g	—	20.8
Iron, mg	9	15
Zinc, mg	12	19
Phosphorus, μg	700	1200
Selenium, μg	70	75
Thiamin, mg	1.4	1.6
Riboflavin, mg	1.6	1.8
Niacin, mg equivalent	17	20
Pyridoxine, mg	2.0	2.2
Vitamin B-12, μg	2.8	2.6
Ascorbic acid, mg	120	100
α-Tocopherol, mg	19	12
Folic acid, μg	500	300
Iodine, μg	290	200
Potassium, g	5.1	1.1
Magnesium, mg	310	124
Copper, mg	1.3	0.3
Calcium, mg	1000	294

[1]The supplement consisted of ground peanuts, dried skim milk, vegetable fat, sugar, multivitamin-mineral premix (Nutriset, France; www.nutriset.fr). RDA, Recommended Dietary Allowance [Institute of Medicine (15)].

TABLE 2
Characteristics of participants in the Breastfeeding, Antiretrovirals, and Nutrition study at the initial time point[1]

	Treatment group							
	Control		LNS		ARV		ARV + LNS	
Characteristic	n	Value	n	Value	n	Value	n	Value
Age, y	176	25.5 (22.9–29.8)[2]	183	26.2 (22.2–29.9)	85	27.0 (24.0–29.4)	90	25.0 (22.9–30.0)
Postprimary education, %	177	36.7	185	37.8	85	35.3	90	32.2
Literacy, %	172	78.5	179	75.4	81	76.5	85	77.6
Married, %	177	90.4	185	90.3	85	89.4	90	92.2
Vaginal delivery, %	177	96.6	185	95.7	85	96.5	90	94.4
Anthropometric measurements								
Height, cm	177	157 (154–160)	185	155 (152–159)	85	157 (154–160)	90	157 (154–161)
Weight, kg	177	55.5 (50.0–60.3)	185	54.4 (50.3–59.1)	85	54.6 (50.5–60.2)	90	54.5 (50.2–60.4)
BMI, kg/m^2	177	22.4 (20.7–24.1)	185	22.3 (20.9–24.2)	85	22.3 (20.6–24.3)	90	22.2 (20.8–23.7)
Laboratory measurements								
Hemoglobin, g/L	177	122 (111–131)	183	121 (111–131)	85	121 (107–126)	90	120 (111–131)
CD4 count, cells/μL	159	465 (319–665)	170	500 (337–738)	78	616 (439–780)	85	607 (412–796)

[1]ARV, antiretroviral; LNS, lipid-based nutrient supplement.
[2]Median; IQR in parentheses (all such values).

ultraperformance liquid chromatography–tandem mass spectrometry (21). Briefly, samples were subjected to protein precipitation, and nonpolar constituents were removed by liquid-liquid extraction before analysis. Thiamin monophosphate (TMP), thiamin pyrophosphate (TPP), and free thiamin were measured by HPLC-fluorescence detection after precolumn derivatization by using the thiochrome method as described previously (22, 23) with a few modifications. An Agilent Zorbax Eclipse Plus C$_{18}$ column (4.6 × 150 mm, 5 μm) was used with an 8-min gradient of 0.15 mol K$_2$HPO$_4$/L (pH 7.0; A) and methanol (B) as follows: 0 min, 15% B; 1–3 min, 20% B; 3–6 min, 50% B; and 7–8 min, 15% B at a flow rate of 1.5 mL/min, which allowed the simultaneous detection of TPP, TMP, and thiamin derivatives. Vitamin B-12 concentrations were measured by using the IMMULITE solid-phase, competitive chemiluminescent enzyme immunoassay (24). Thiamin was calculated as the sum of combined free thiamin, TMP, and TPP concentrations based on molecular weights: thiamin = free thiamin + (TMP × 0.871) + (TPP × 0.707). Riboflavin was calculated as the

TABLE 3
Median concentrations (and IQRs) of thiamin, riboflavin, nicotinamide, pyridoxal, and vitamin B-12 in breast milk from HIV-infected women assigned to 1 of 4 treatment arms in the Breastfeeding, Antiretrovirals, and Nutrition study[1]

	Treatment group				P[2]		
Vitamin	Control (n = 177)	LNS (n = 185)	ARV (n = 85)	ARV/LNS (n = 90)	LNS	ARV	LNS × ARV
Thiamin,[3] μg/L					0.44	0.82	0.53
2 or 6 wk	176 (130–220)[4]	178 (144–212)	169 (123–215)	173 (134–229)			
24 wk	199 (160–238)	208 (174–241)	202 (176–238)	201 (165–242)			
Riboflavin,[5] μg/L					<.001	0.071	0.015
2 or 6 wk	100 (71–147)	133 (87–184)	104 (74–132)	114 (81–148)			
24 wk	94 (69–124)	129 (88–168)	95 (66–127)	102 (67–163)			
Nicotinamide, μg/L					0.001	<0.001	0.12
2 or 6 wk	430 (244–689)	566 (374–793)	386 (248–519)	404 (253–708)			
24 wk	219 (124–358)	281 (169–457)	161 (102–268)	188 (97–315)			
Pyridoxal, μg/L					<0.001	0.006	0.12
2 or 6 wk	62 (40–99)	84 (57–109)	56 (39–80)	64 (50–89)			
24 wk	113 (82–150)	142 (100–190)	110 (77–152)	120 (83–161)			
Vitamin B-12, μg/L					0.0018	0.0036	0.16
2 or 6 wk	0.33 (0.22–0.59)	0.41 (0.26–0.81)	0.29 (0.20–0.55)	0.30 (0.23–0.49)			
24 wk	0.24 (0.19–0.35)	0.32 (0.23–0.49)	0.23 (0.19–0.33)	0.28 (0.21–0.39)			

[1]Main effects and interactions of LNS and ARV were tested by ANOVA. A mixed-model repeated-measures analysis was used to test for the main effect of time and interactions between treatment variables and time (no interactions with time were significant). ARV, antiretroviral; LNS, lipid-based nutrient supplement; n, number of samples.
[2]P values represent both time points because of no significant LNS × ARV × time interaction.
[3]Thiamin = thiamin + (thiamin monophosphate × 0.871) + (thiamin pyrophosphate × 0.707).
[4]Median; IQR in parentheses (all such values).
[5]Riboflavin = riboflavin + (flavin adenine dinucleotide × 0.479).

sum of the combined free riboflavin and FAD concentrations, also based on molecular weights: riboflavin = free riboflavin + (FAD × 0.479) (25, 26).

Statistical analysis

The outcomes were concentrations of thiamin, riboflavin, nicotinamide, pyridoxal, and vitamin B-12 in breast milk within the 4 defined groups (antiretrovirals + LNSs, antiretrovirals, LNSs, and control). SAS statistical software 9.4 (SAS Institute) was used for all statistical analyses. Logarithmic transformations were performed on thiamin, riboflavin, nicotinamide, and vitamin B-12, and square root transformations were performed on pyridoxal concentrations to normalize the distributions. The original hypothesis considered only outcomes due to LNS supplementation. However, preliminary data showed significant effects of antiretrovirals for some of the micronutrients; therefore, possible negative interactions with antiretrovirals were also analyzed. Mixed-model repeated-measures ANOVA (MIXED procedure) was used to fit a 3-factor model, which included LNSs and antiretrovirals as between-subject main effects, time as a within-subject main effect, all 2- and 3-factor interactions, and a random effect of subject, assuming an unstructured covariance matrix. Because no significant interactions with time were observed, the 2 time points were pooled to assess the effect of treatment. For outcomes with a significant interaction ($P < 0.05$) between LNSs and antiretrovirals, pairwise comparisons were performed, adjusted for multiple comparisons with Tukey-Kramer's test; otherwise, only main effects were examined. Breast-milk B vitamin concentrations were also compared with the values used by the Institute of Medicine to set recommended intakes for infants from 0 to 6 mo of age (15). P values <0.05 were considered to be statistically significant.

RESULTS

Maternal characteristics at initial visit

No differences were observed in the characteristics of study participants among the groups at the initial time point (**Table 2**).
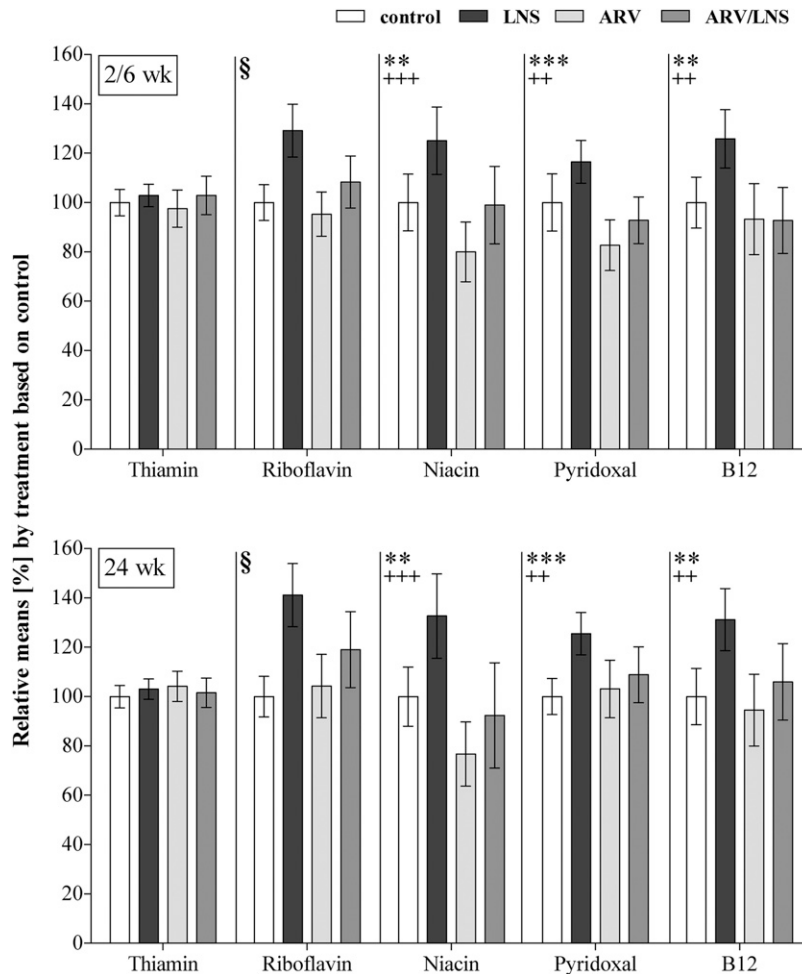

FIGURE 1 Relative mean concentrations and 95% CIs of B vitamins in the treatment groups. Main effects (LNS − no LNS; ARV – no ARV) and interactions between LNS, ARV, and time were tested by mixed-model repeated-measures ANOVA. No interactions with time were significant. Significant main effects of LNS: **$P < 0.01$, ***$P < 0.001$. Significant main effects of ARVs: ++$P < 0.01$, +++$P < 0.001$. §Significant ARV × LNS interaction: pairwise comparisons were adjusted for multiple comparisons with Tukey-Kramer's test, LNS significantly increased riboflavin concentrations ($P < 0.0001$ when compared with the control and ARV groups; $P = 0.015$ when compared with the ARV + LNS group), and no significant differences were observed between the 3 remaining groups. Control group: $n = 177$; LNS group: $n = 185$; ARV group: $n = 85$; ARV + LNS group: $n = 91$. ARV, antiretroviral; LNS, lipid-based nutrient supplement.

The average BMI was within the normal range. Overall compliance with antiretroviral treatment and supplementation was high. Mothers self-reported that they took their prescribed antiretroviral treatment 89% of the time and LNS supplement 92% of the time, based on adherence reports collected over 5 follow-up visits. The self-reported frequency of EBF was 96% at 21 wk postpartum (8, 11).

Overall treatment effects and time interaction

The results of the mixed model showed that the concentrations of all vitamins changed over time ($P < 0.012$ for all). However, none of the interactions involving time were significant, which indicated that the effects of antiretrovirals and LNSs at 2 or 6 wk were not different from the effects at 24 wk; therefore, we pooled the data from the 2 time points for the statistical analysis. A positive overall effect of LNSs on all vitamins, except thiamin, and a negative overall effect of antiretrovirals on nicotinamide, pyridoxal, and vitamin B-12 were observed. The LNS × antiretroviral interaction was significant for riboflavin only ($P = 0.015$; **Table 3**).

Treatment effects at 2 or 6 and 24 wk

Concentrations of the nutrients in milk varied widely among women in every treatment group, independently of the time of collection. All women assigned to receive LNSs started at or very close to delivery. Interactions between antiretrovirals and LNSs were observed only for riboflavin (Table 3). The main effects of LNSs and antiretrovirals were significant for nicotinamide, pyridoxal, and vitamin B-12, but not for thiamin (**Figure 1**). Whereas LNS consumption resulted in significantly higher concentrations, antiretroviral treatment lowered the amounts of these 3 vitamins in breast milk. Riboflavin concentrations increased with LNSs when compared with antiretrovirals, antiretrovirals + LNSs, and the control treatment ($P < 0.016$ for all), but were not influenced by antiretrovirals regimen alone. No significant differences were observed between the antiretrovirals, antiretrovirals + LNSs, and control groups. Neither LNS nor antiretroviral treatment affected thiamin concentrations. The consumption of antiretrovirals with LNSs eliminated the positive effects of LNS supplementation on all vitamins but thiamin, i.e., the resulting milk concentrations were reduced to the concentration of the control group.

Comparison of milk B vitamin concentrations at 24 wk with values used to set the AI for infants aged 0–6 mo

In comparison with the concentrations used to estimate the AI for infants aged 0–6 mo (**Table 4**) (15), only 50–56% of the samples met the estimated value for thiamin concentration in milk regardless of treatment group. For pyridoxal, 60% of the samples of the LNS group reached the values used to set the AIs, whereas only 36–44% of the samples were adequate in the remaining treatment groups. In contrast, <2% of samples met the AI milk values for riboflavin (0.6–1.6%) and nicotinamide (0–1.6%) in any group; 16–22% met vitamin B-12 assumptions, which increased to 34% when mothers were given LNSs alone.

DISCUSSION

On the basis of strong evidence of the benefits of EBF during the first 6 mo of life, it is crucial to understand how factors such as maternal micronutrient intake, status, supplementation, and antiretroviral therapy affect breast-milk concentrations of nutrients during the period of lactation. In areas where dietary intake alone may not be sufficient to guarantee an adequate supply of vitamins in milk, maternal supplementation may help to ensure adequate nutrition of the infant. LNSs have been used historically in the clinical setting for the treatment of severe acute malnutrition in children and are currently being evaluated in trials aimed at improving birth and health outcomes in infants and children with moderate malnutrition (18, 27). In this trial, LNSs mitigated maternal weight loss, even in women receiving antiretrovirals (11).

These results suggest that the usual maternal intake of all of the vitamins measured is insufficient, given the observation that <1% of control samples reached the concentration used to set the AI values for riboflavin and nicotinamide and only 16–50% of samples met the AI values for any of the other B vitamins. However, given that AIs are based on studies with small sample sizes collected under unclear and variable conditions (12), the true reference nutrient contents of breast milk and subsequently the requirements of infants during the first 6 mo of life remain somewhat uncertain.

Maternal LNS supplementation increased breast-milk concentrations of riboflavin, nicotinamide, pyridoxal, and vitamin B-12. However when concentrations were compared with the values used to set the AI (15, 28), only 2–60% of the participants receiving LNSs alone met the AI estimates across all the vitamins measured. Therefore, only a limited amount of the micronutrients in maternal LNSs get transferred to the infant

TABLE 4
Percentage of samples meeting the values used to set the AI for each vitamin, by collection time and treatment group[1]

Vitamin	AI, μg/L	Control (*n* = 177)	LNS (*n* = 185)	ARV (*n* = 85)	ARV + LNS (*n* = 90)
Thiamin,[2] %					
2 or 6 wk	200	35	35	31	36
24 wk	200	50	56	52	50
Riboflavin,[3] %					
2 or 6 wk	350	0	3.2	0	0
24 wk	350	0.6	1.6	1.2	1.1
Nicotinamide, %					
2 or 6 wk	1800	2.8	5.4	1.2	1.1
24 wk	1800	0	1.6	0	1.1
Pyridoxal, %					
2 or 6 wk	130	9.0	17	5.9	7.8
24 wk	130	36	60	44	41
Vitamin B-12, %					
2 or 6 wk	0.42	35	47	33	33
24 wk	0.42	16	34	17	22

(The header "Treatment group" spans the Control, LNS, ARV, and ARV + LNS columns.)

[1]AI, Adequate Intake; ARV, antiretroviral; LNS, lipid-based nutrient supplement; *n*, number of samples.
[2]Thiamin = thiamin + (thiamin monophosphate × 0.871) + (thiamin pyrophosphate × 0.707).
[3]Riboflavin = riboflavin + (flavin adenine dinucleotide × 0.479).

via breast milk and may not fulfill the infant's requirements. Duggan et al. also observed significantly higher vitamin B-12 concentrations in breast milk collected at 6 wk, but not at 3 or 6 mo postpartum after a daily maternal supplement consumption of 50 μg/d during pregnancy through 6 wk postpartum (29). This supplementation regimen resulted in a median breast-milk vitamin B-12 concentration of only 136 pmol/L (0.18 μg/L) vs. 87 pmol/L, less than half of the value assumed to set the AI. Even a daily maternal supplementation of 250 μg/d during pregnancy up to 3 mo postpartum (30) resulted in a median vitamin B-12 concentration at 3 mo of 235 pmol/L (0.32 μg/L) vs. 170 pmol/L, which indicated a limitation of vitamin transfer into breast milk. Recommendations regarding antiretroviral use during lactation were introduced in the 2010 WHO guidelines in response to evidence of a reduced risk of postnatal transmission of HIV through breastfeeding when antiretrovirals are given to either the HIV-infected mother or the HIV-exposed infant (1). Thus, it is important to understand the effect of these treatments on the concentrations of nutrients in breast milk and the potential effect on the exclusively breastfed infant. Some antiretrovirals given to the mother do appear in breast milk. In a study conducted in Botswana, the concentrations of nevirapine, lamivudine, and zidovudine were 0.67, 3.34, and 3.21 times, respectively, the concentrations in maternal serum (31). Whereas these drugs were also found in breast milk analyzed within the Breastfeeding, Antiretrovirals, and Nutrition study, only lamivudine was detected in infant plasma (32). However, it has been shown that infants exposed to the maternal antiretroviral regimen through breast milk developed HIV-1 drug resistance, which indicated the need for close monitoring of the infants' HIV status and to consider the maternal regimen when deciding on infant treatment (33). These findings are important in the context of Option B+, which provides lifelong antiretroviral therapy to pregnant and/or lactating women as soon as HIV is diagnosed, regardless of their baseline CD4 count, as recommended by the WHO (34, 35). Although Option B+ aids considerably in the Prevention of Mother-to-Child Transmission program in resource-limited countries with a high prevalence of HIV, it has been shown that micronutrients such as vitamin D and B-12 can be negatively affected by antiretrovirals based on lower plasma concentrations (36–38). These observations call attention to the need to measure effects of Option B+ on maternal and infant vitamin status.

LNSs increased all B vitamin concentrations in breast milk, except thiamin. Antiretroviral treatment without LNS supplementation negatively affected the concentrations of nicotinamide, pyridoxal, and vitamin B-12. When LNS was taken with antiretroviral treatment, the positive effect of supplementation was offset by the negative effect of the antiretrovirals on nicotinamide, pyridoxal, and vitamin B-12, which resulted in vitamin concentrations that were comparable with those in the control group. Even though antiretroviral treatment alone did not affect milk riboflavin concentrations, the combination of antiretrovirals with LNSs prevented LNS-induced increases in this vitamin. These results suggest that antiretroviral treatment negatively influences the uptake or transfer of certain B vitamins in breast milk and that LNS supplementation of lactating women receiving antiretrovirals may be beneficial to breast-milk vitamin concentrations and subsequently to infants who are exclusively breastfed. It remains to be determined whether the timing of taking supplements and antiretrovirals might improve the benefits of micronutrient supplementation.

Even though the number of milk samples analyzed in the current study is extensive, they may not be representative of 24-h milk production, because the samples were not obtained by emptying the breast. No information is available about the timing of the last infant feed or LNS consumption, but usually there is relatively little variability in B vitamin concentrations during a feed. In addition, because our initial samples were from 2 or 6 wk, the actual effect of the treatments from 0 to 2 wk and 0 to 6 wk is not reflected in the analysis. Nevertheless, we concluded that LNS supplementation had a positive effect on breast-milk concentrations of all B vitamins analyzed, except thiamin, whereas antiretroviral treatment affected B vitamin uptake into breast milk. Additional studies are necessary to gain a better understanding of the interactions between maternal antiretroviral treatment and micronutrient supplementation and their long-term effects on breast-milk vitamin concentrations and maternal and infant status. In addition, more information is desirable about the effect of different levels of supplemental nutrients, and the optimal timing of supplementation during pregnancy or lactation, in healthy women and in the context of HIV infection.

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