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Anemia and Micronutrient Status during Pregnancy, and Their Associations with Obstetric and Infant Outcomes among HIV-Infected Ugandan Women Receiving Antiretroviral Therapy

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ABSTRACT

Background: Women living with HIV (WLHIV) are at higher risk of micronutrient deficiencies and adverse health outcomes. There are limited data on the burden or sequelae of micronutrient deficiencies among pregnant WLHIV receiving antiretroviral therapy (ART).

Objectives: We aimed to examine anemia and vitamin B-12, folate, and vitamin D deficiencies, and their associations with obstetric and infant outcomes, among pregnant WLHIV initiating combination antiretroviral therapy (cART) in rural Uganda.

Methods: This was a prospective analysis among pregnant WLHIV (12–28 weeks of gestation) in PROMOTE-Pregnant Women and Infants (PIs), a randomized trial comparing the effects of protease inhibitor (PI)-based ART with those of a non-PI-based ART on placental malaria risk. We conducted a substudy on the burden of anemia [trimester 1/3: hemoglobin (Hb) <11.0 g/dL; trimester 2: Hb <10.5 g/dL; $n = 367$] and micronutrient deficiencies ($n = 127$) in pregnant WLHIV and their associations with obstetric and infant outcomes. Hb was measured by cyanmethemoglobin, vitamin B-12 and folate were measured via electrochemiluminescence, and vitamin D was measured by ELISA. Linear and binomial regression were used to evaluate associations between micronutrient status during pregnancy and perinatal outcomes.

Results: 26.8% women were anemic, 30.2% were vitamin B-12 insufficient (<221.0 pmol/L), 66.1% were folate insufficient (<13.5 nmol/L), and 65.4% were vitamin D insufficient (<30.0 ng/mL) at enrollment. Anemia during pregnancy was associated with a greater risk of small for gestational age (SGA) (RR: 1.88; 95% CI: 1.28, 2.77; $P = 0.001$); each 1-g/dL decrease in Hb was associated with greater risk of SGA (RR: 0.76; 95% CI: 0.65, 0.90; $P = 0.001$). Multivariate models showed that increased vitamin D concentrations predicted lower risk of infant wasting (WLZ < -2; RR: 0.94; 95% CI: 0.89, 0.99; $P = 0.04$). Multivariate models also indicated that maternal vitamin B-12 and folate concentrations at enrollment predicted maternal ($P < 0.001$) and infant ($P = 0.02$) concentrations postpartum.

Conclusions: Anemia and micronutrient deficiencies are associated with a variety of adverse obstetric and infant outcomes and are an important public health concern in perinatal WLHIV on cART and their children. This trial was registered at clinicaltrials.gov as NCT00993031. *Curr Dev Nutr* 2020;4:nzaa075.

Keywords: anemia, vitamin D, vitamin B-12, folate, sub-Saharan Africa, pregnant, postpartum, micronutrient, AIDS

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Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.
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Abbreviations used: ART, antiretroviral therapy; cART, combination antiretroviral therapy; Hb, hemoglobin; LAZ, length-for-age *z* score; LMP, last menstrual period; PI, protease inhibitor; SGA, small for gestational age; TS, trimethoprim-sulfamethoxazole; WAZ, weight-for-age *z* score; WLHIV, women living with HIV; WLZ, weight-for-length *z* score.

Introduction

Micronutrient deficiencies are common in individuals living with HIV. They have been associated with increased risk of HIV disease progression, morbidity, and mortality (1–5). In pregnancy, women living with HIV (WLHIV) are at increased risk of anemia, micronutrient deficiencies, and adverse obstetric outcomes (5). The etiology of anemia in pregnant WLHIV is multifactorial and may be attributed to iron de-

ficiency or anemia of inflammation from HIV, opportunistic infections, or other infections, e.g., malaria or hookworm. Other micronutrient deficiencies, such as vitamin B-12, folate, and vitamin D deficiencies, may also contribute to the risk of anemia and adverse HIV-related outcomes (6, 7). Lower vitamin B-12 and folate status have been associated with increased HIV disease progression (8, 9), and randomized trials have found that B-vitamin supplementation decreases the risk of anemia, adverse pregnancy outcomes, opportunistic infections, and weight loss in antiretroviral therapy (ART)-naïve pregnant WLHIV (10–13). Vitamin D is essential for both adaptive and innate immune responses (14), and vitamin D deficiency is common in HIV-infected populations (8, 15, 16). Studies have also found high prevalence of low vitamin D concentrations and a significant association with wasting among HIV-exposed, HIV-uninfected infants (5, 17). In order to better characterize and address these micronutrient deficiencies, it is critical to document their extent in settings with high overlapping burdens of HIV and malnutrition.

Despite the critical role that micronutrients play in health outcomes during the first 1000 days of life, there are limited data on the burden of micronutrient deficiencies, or their associations with risk of adverse obstetric and infant outcomes, among pregnant WLHIV initiating ART and their children. Research to date has primarily been conducted among ART-naïve pregnant WLHIV (18–20) before ART or combination antiretroviral therapy (cART) was widely available or the WHO standard of care for all pregnant and lactating women (21–26).

Further, as cART is rapidly scaled up worldwide, there is a growing population of HIV-exposed, uninfected children. Emerging evidence suggests that children who are exposed to HIV in utero may also be at higher risk of malnutrition, poor cognitive development morbidity, and mortality, even if they are not HIV-infected (1, 27–29). Previous studies among pregnant WLHIV on cART have noted that HIV-exposed, breastfed infants experienced increased risk of infectious disease morbidity and more frequent hospital admissions compared with HIV-unexposed, breastfeeding infants (30, 31). Although cART is the gold standard for prevention of mother-to-child transmission of HIV, there are limited data on the potential impact of additional strategies (e.g., adjunct micronutrient supplementation) to help reduce morbidity and mortality and support nutrition and health early in life in HIV-exposed infants.

Given that pregnant WLHIV are at higher risk of both micronutrient deficiencies and adverse pregnancy outcomes, micronutrient supplementation may improve obstetric and infant outcomes among this population. However, the limited prospective data on mothers and infants receiving ART constrain the development of nutritional guidelines tailored for this vulnerable population. There are no specific WHO guidelines to support the nutritional needs of pregnant WLHIV or their children—beyond breastfeeding recommendations, standard iron and folic acid supplementation, adequate diet (i.e., dietary intake of micronutrients at 1-RDA level), and hemoglobin (Hb) assessment. The current WHO guidelines (2003) remain the same for pregnant women regardless of HIV status, and state that further research is needed before specific nutritional recommendations can be made for pregnant WLHIV and their children (32, 33). As such, prospective data on the burden and sequelae of micronutrient deficiencies among pregnant WLHIV who are initiating cART are urgently needed to inform WHO guidelines and to support the health of WLHIV and their children.

Therefore, in this article, we examine the burden of anemia, and micronutrient deficiencies of vitamin B-12, folate, and vitamin D, and their associations with obstetric and infant outcomes at birth, among pregnant WLHIV initiating cART in rural Uganda. Specifically, we hypothesized that maternal anemia and micronutrient deficiencies early in pregnancy would be associated with an increased risk of poor obstetric outcomes, poor infant growth, and suboptimal nutritional status.

Methods

Study design and population

This prospective analysis was conducted among pregnant WLHIV enrolled in the PROMOTE-Pregnant Women and Infants (PIs) study, a randomized clinical trial in Uganda (NCT00993031) (34, 35). This open-label, single-site, randomized controlled trial was designed to test the hypothesis that pregnant women receiving a protease inhibitor (PI)-based ART regimen would have a lower risk of placental malaria than pregnant women receiving a non-PI-based ART regimen (35). Women were randomly assigned to receive lopinavir/ritonavir-based or efavirenz-based ART. The study was conducted from December 2009 to March 2013 in Tororo, a district in rural eastern Uganda.

The design of this trial has been previously described (34, 35). Briefly, women were recruited from the Tororo District Hospital antenatal clinic, The AIDS Support Organization, and other health centers in the district. Women were eligible for inclusion in the parent trial (PROMOTE) if they were ≥16 y of age, infected with HIV-1, lived within 30 km of the study site, and were pregnant (12–28 weeks of gestation), as assessed by last menstrual period (LMP) with confirmation by ultrasound. Women were excluded if they had ever received cART, received single-dose nevirapine within the past 2 y, exhibited dose-limited toxicity to trimethoprim-sulfamethoxazole (TS) within the past 2 wk, received any medications that were contraindicated, or had cardiac abnormalities, WHO stage IV diseases, severe anemia (Hb <7.5 g/dL), or abnormal laboratory values at screening. All women provided written informed consent.

We conducted a substudy to examine the prevalence of maternal anemia ($n = 367$) and micronutrient deficiencies ($n = 127$; vitamin B-12, folate, and vitamin D) in pregnant WLHIV and their associations with obstetric and infant outcomes within the PROMOTE trial. Participants were selected to participate in the substudy if a sufficient aliquot quantity was available, and if they had been enrolled before the commencement of a lipid-based nutrient supplementation substudy (36).

Ethics

The study protocol was reviewed and approved by the Makerere University School of Medicine Research and Ethics Committee, the Uganda National Council for Science and Technology, and the University of California San Francisco Committee on Human Research.

Follow-up procedures

Demographic data and clinical HIV, medical, and obstetric history data were collected at baseline by trained study staff members. HIV infection was determined by a rapid HIV antibody test (Determine, Inverness Medical Japan Co.) and confirmatory test (Stat-Pak, Chembio Diagnostic Systems, Inc.). As part of the trial, pregnant women were

enrolled at 14–28 weeks of gestation and randomly assigned to receive either PI-based ART or non-PI-based ART (i.e., zidovudine, lamivudine, and efavirenz compared with zidovudine, lamivudine, lopinavir, and ritonavir). Participants also received daily TS (cotrimoxazole) as malaria prophylaxis if they were not already receiving it before study enrollment, mebendazole, and insecticide-treated bed nets. In addition, at each monthly visit, all women received 5 wk of prenatal multivitamins (Pregnacare Original, Vitabiotics) containing 10 μg vitamin D, 6 μg vitamin B-12, 17 mg Fe, and 400 μg folate as well as an additional tablet containing 200 mg ferrous sulfate; their use was encouraged but not assessed.

Maternal height was measured using a Seca 206 measuring tape mounted on the wall (to the nearest 0.1 cm) and weight was measured using a Seca 876 scale (to the nearest 500 g). LMP was used to estimate gestational age, and gestational age was confirmed by ultrasound. Final gestational age was determined based on ultrasound if the discrepancy between LMP and ultrasound was greater than predetermined criteria (i.e., 1 wk in the first trimester, 2 wk in the second trimester, or 3 wk in the third trimester). Ultrasound was used as the method to date 48.1% of the pregnancies included in these analyses [50.5% of second-trimester pregnancies ($n = 105$) and 43.4% of third-trimester pregnancies ($n = 53$)].

Enrolled women returned to the study clinic for scheduled visits every 4 wk and for any acute health care needs, including adverse events. Women received free antenatal care in accordance with standard of care guidelines of the Ministry of Health of Uganda. At monthly study visits, trained study counsellors collected data on health outcomes and maternal anthropometry, and nutritional counseling was provided based on the most recent WHO and national infant feeding recommendations (37). Women were counseled to breastfeed exclusively for at least the first 6 mo and to continue breastfeeding through 1 y postpartum. At each study visit, a 5-wk supply of multivitamins, cART, and TS was provided to participants.

Infant anthropometry was evaluated by trained study staff immediately after birth for infants delivered at the study hospital. If a delivery occurred at home or at another health facility, measurements were taken at the first postpartum clinic visit. Trained staff used standardized protocols and calibrated instruments for infant anthropometric measurements. Infant weight was measured using a digital Seca 354 scale (to the nearest 10 g). Infant length was assessed using a length board that was made locally. Head circumference was measured using nonstretchable tape (Seca 212; to the nearest 0.1 cm).

All infants received prophylactic ART at birth in accordance with Ugandan Ministry of Health guidelines: zidovudine for 7 d until November 2010, when nevirapine syrup (10 mg/mL) was provided from birth for 6 wk, and TS daily from 6 wk of age until 6 wk after breastfeeding cessation.

Laboratory investigations

The laboratory protocols and analyses conducted in the parent trial have previously been described (34, 35). Briefly, maternal CD4 T-cell counts were assessed at screening, during pregnancy (12 and 24 wk after enrollment), at delivery, and during the postpartum period (24 and 48 wk). Maternal PCR testing for HIV-1 RNA was performed 8 wk after the start of ART, at delivery, and during the postpartum period (8, 24, and 48 wk postpartum). HIV disease progression was classified using the 2007 WHO criteria (38). Infant HIV infection was evaluated at birth via HIV-1 DNA PCR (Cobas Amplicor, Roche Diagnostics).

Approximately 10 mL of blood were drawn from mothers or infants at each time point in both EDTA-coated and plain vacutainers (BD Biosciences) that were stored on ice until separation in a refrigerated centrifuge within 4 h. Hb concentrations were measured by cyanmethemoglobin and complete blood counts were analyzed on whole-blood samples. Samples were separated, and plasma and serum samples were stored at or below −80°C until analysis. Serum vitamin B-12 and folate concentrations were measured via electrochemiluminescence, whereas vitamin D concentrations were measured by ELISA.

Maternal micronutrient status

Maternal anemia was defined using trimester-specific cutoffs based on WHO criteria (i.e., first trimester: Hb <11.0 g/dL; second trimester: Hb <10.5 g/dL; and third trimester: Hb <11.0 g/dL) (39, 40). Vitamin B-12 deficiency (<148.0 pmol/L) and insufficiency (<221.0 pmol/L) and folate deficiency (<6.8 nmol/L) and insufficiency (<13.5 nmol/L) were defined using established CDC criteria (41, 42). Vitamin D deficiency (<20.0 ng/mL) and insufficiency (<30.0 ng/mL) were defined based on clinical practice guidelines of the Endocrine Society (43).

Obstetric and infant outcomes

Obstetric outcomes included gestational age at delivery, preterm birth (<37 weeks of gestation), birth weight, low birth weight (<2500 g), composite preterm birth and low birth weight, and small for gestational age (SGA). SGA was defined as below the 10th percentile of gestational age, using sex-specific INTERGROWTH criteria (44).

Infant micronutrient status and anthropometric outcomes were evaluated at birth. Micronutrient status outcomes included Hb, vitamin B-12, folate, and vitamin D concentrations; anemia (<11.0 g/dL), folate deficiency and insufficiency (<6.8 and <13.5 nmol/L, respectively) (41, 42), vitamin B-12 deficiency and insufficiency (<148.0 and <221.0 pmol/L, respectively) (41, 42), and vitamin D deficiency and insufficiency (<20.0 and <30.0 ng/mL, respectively) (43). Standard WHO methods were used to calculate length-for-age (LAZ), weight-for-age (WAZ), and weight-for-length (WLZ) z scores (45), and to define stunting (LAZ < -2), underweight (WAZ < -2), and wasting (WLZ < -2). Infant ponderal index was defined as weight (g) divided by length (cm) cubed (g/cm^3).

Statistical analyses

Linear and binomial regression models (i.e., Proc GenMod with log-link function) were used to evaluate associations of maternal micronutrient status during pregnancy with obstetric and infant outcomes. Variables that were not normally distributed were ln transformed to achieve normality before analysis. Nonlinearity of observed associations was examined nonparametrically, using restricted cubic splines (46, 47). Confounding was evaluated and adjusted for using the approach described by Greenland (48), in which all suspected or known risk factors for the outcome which resulted in a >10% change in the effect estimated were retained in models. Final models were adjusted for the antiretroviral intervention, and gestational age, maternal age, BMI, and log CD4 T-cell counts at enrollment. The missing indicator method was used to

retain observations with missing covariate data (49). Statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Inc.).

Results

Study population

Table 1 presents baseline characteristics of the study population. Anemia data were available on the entire cohort; a total of 127 mother–infant pairs were included in the micronutrient substudy of vitamin B-12, folate, and vitamin D. There were no significant differences in baseline characteristics of the micronutrient substudy cohort ($n = 127$) compared with women in the entire cohort ($n = 367$), and there were no significant differences in baseline characteristics by study arm within the subsample. At enrollment, >90% of women were HIV WHO stage I and 58.3% of women presented with CD4 T-cell counts >350 cells/μL. Median gestational age at enrollment was 22.0 wk (IQR: 18.0–24.7 wk); 96.9% of pregnant women were enrolled during the second trimester of pregnancy (T1: 1.6%; T2: 96.9%; T3: 1.6%). Mean maternal age was 30.0 y (median: 30.0 y; IQR: 26.8–34.4 y) and >95% were multiparous.

Maternal anemia and micronutrient status

A total of 26.8% of women included in the micronutrient substudy were anemic (trimester 1: Hb <11.0 g/dL; trimester 2: Hb <10.5 g/dL; trimester 3: Hb <11.0 g/dL) at enrollment, with median Hb concentrations of 11.1 g/dL (IQR: 10.3–11.9 g/dL). Maternal B-vitamin deficiencies were common at the first prenatal visit: 66.1% of women were folate insufficient (<13.5 nmol/L), 7.1% were vitamin B-12 deficient (<148.0 pmol/L), and 30.2% were vitamin B-12 insufficient (<221.0 pmol/L) at enrollment. The prevalence of vitamin D insufficiency was also high, with 26.0% of women with 25-hydroxyvitamin D [25(OH)D] concentrations <20.0 ng/mL and 65.4% with 25(OH)D concentrations <30.0 ng/mL at enrollment (Table 1).

Pregnancy and infant outcomes

Table 2 presents obstetric outcomes and infant characteristics. A total of 15.0% of infants were born preterm (<37 wk), 12.2% were born low birth weight (<2500 g; median: 2900 g; IQR: 2700–3240 g), and 21.1% were SGA. A total of 21.2% of infants were stunted (LAZ < −2), 10.6% were underweight (WAZ < −2), and 6.4% were wasted (WLZ < −2). A total of 2.7% of infants were anemic at birth (Hb <11.0 g/dL for 0–6 mo; median: 15.5 g/dL; IQR: 14.0–17.0 g/dL). None of the infants were vitamin B-12 deficient or insufficient at birth. However, 44.4% of infants were folate insufficient (<13.5 nmol/L), and 82.9% and 57.1% were vitamin D insufficient [25(OH)D <30.0 ng/mL] or deficient [25(OH)D <20.0 ng/mL], respectively.

Maternal hematological status and pregnancy and infant outcomes

Table 3 presents the associations of maternal Hb concentrations and anemia at enrollment with obstetric and infant outcomes. Maternal anemia at enrollment predicted a 2-fold greater risk of SGA (RR: 1.88; 95% CI: 1.28, 2.77; $P = 0.001$) than in women who were not anemic, with a 24% lower risk of SGA per unit (g/dL) increase in baseline Hb (RR: 0.76; 95% CI: 0.65, 0.90; $P = 0.001$) concentrations. There were no significant associations of maternal Hb concentrations or anemia with infant anthropometric outcomes, including length, LAZ, WAZ, WLZ, stunting, underweight, wasting, ponderal index, or head circumference.

Maternal micronutrient status and pregnancy and infant outcomes

Table 4 presents the associations of maternal vitamin B-12, folate, and vitamin D concentrations at enrollment with obstetric and infant outcomes. Higher maternal vitamin D concentrations were associated with lower risk of wasting (WLZ < −2; RR: 0.94; 95% CI: 0.89, 0.99; $P = 0.04$), after adjusting for antiretroviral treatment arm, gestational age at enrollment, maternal age, BMI, and log CD4 T-cell counts, with a 6% lower risk per 1-ng/mL increase in baseline vitamin D concentrations. However, higher maternal vitamin D concentrations were also associated with an increased risk of preterm birth (<37 wk; RR: 1.05; 95% CI: 1.00, 1.11; $P = 0.04$) in multivariate analyses. Higher maternal vitamin B-12 concentrations at enrollment were associated with increased risk of low birth weight (RR: 4.78; 95% CI: 1.35, 16.93; $P = 0.02$); lower LAZs ($\beta \pm$ SE: -0.59 ± 0.26, $P = 0.02$), WAZs ($\beta \pm$ SE: -0.45 ± 0.20, $P = 0.03$), and head circumference ($\beta \pm$ SE: -0.68 ± 0.34, $P = 0.04$); and increased risk of stunted (LAZ < −2; RR: 2.27; 95% CI: 1.05, 4.93; $P = 0.04$) and underweight infants (WAZ < −2; RR: 6.39; 1.43, 28.54; $P = 0.02$), in multivariate models adjusting for antiretroviral treatment arm, gestational age at enrollment, maternal age, BMI, and log CD4 T-cell counts. There were no significant associations between maternal folate concentrations and obstetric or infant outcomes.

Maternal baseline micronutrient status and postpartum micronutrient status

Table 5 presents the associations of baseline maternal Hb and micronutrient status with maternal micronutrient status during the postpartum period. Maternal Hb concentrations at baseline significantly predicted higher Hb ($\beta \pm$ SE: 0.51 ± 0.07, $P < 0.0001$) and folate concentrations ($\beta \pm$ SE: 0.11 ± 0.05, $P = 0.04$) during the postpartum period, in multivariate models adjusting for antiretroviral treatment arm, gestational age at enrollment, maternal age, BMI, and log CD4 T-cell counts. Maternal anemia at baseline was associated with significantly lower Hb concentrations ($\beta \pm$ SE: -1.07 ± 0.17, $P < 0.0001$) during the postpartum period. Baseline maternal vitamin B-12 concentrations predicted higher postpartum Hb ($\beta \pm$ SE: 0.72 ± 0.31, $P = 0.02$) and vitamin B-12 ($\beta \pm$ SE: 0.48 ± 0.12, $P < 0.0001$) concentrations, whereas vitamin B-12 insufficiency (<221.0 pmol/L) at baseline was associated with lower Hb ($\beta \pm$ SE: -1.04 ± 0.32, $P = 0.001$) and vitamin B-12 ($\beta \pm$ SE: -0.33 ± 0.11, $P = 0.004$) concentrations. In contrast, maternal vitamin B-12 status at baseline was associated with lower postpartum folate status (folate: $\beta \pm$ SE: -0.45 ± 0.21, $P = 0.03$). Higher baseline folate concentrations were associated with significantly higher folate concentrations ($\beta \pm$ SE: 0.56 ± 0.17, $P = 0.0009$) during the postpartum period; similarly, women with folate deficiency at baseline had significantly lower postpartum folate concentrations ($\beta \pm$ SE: -0.64 ± 0.17, $P = 0.0002$), in multivariate analyses adjusting for antiretroviral treatment arm, gestational age at enrollment, maternal age, BMI, and log CD4 T-cell counts.

TABLE 1 Characteristics of the PROMOTE-Pregnant Women and Infants study population at enrollment[1]

Characteristics	Entire cohort (*n* = 367)	Micronutrient substudy (*n* = 127)
Treatment group		
Lopinavir	184 (50.1)	67 (52.8)
Efavirenz	183 (49.9)	60 (47.2)
Sociodemographic		
Age, y	29.7 [25.6–33.7]	30.0 [26.8–34.4]
Educational level		
None	48 (13.1)	21 (16.5)
Primary	244 (66.7)	88 (69.3)
More than primary	74 (20.2)	18 (14.2)
Gestational age at enrollment, wk	21.3 [17.7–24.9]	22.0 [18.0–24.7]
Trimester 1 (<14)	6 (1.6)	2 (1.6)
Trimester 2 (14 to <28)	353 (96.2)	123 (96.9)
Trimester 3 (≥28)	8 (2.2)	2 (1.6)
Previous pregnancies	3 (2–5)	4 (2–5)
Primiparous	22 (6.0)	6 (4.7)
≥1	345 (94.0)	121 (95.3)
Season of birth		
November–May	208 (56.7)	70 (55.1)
June–October	159 (43.3)	57 (44.9)
HIV-related		
WHO stage		
I	351 (95.6)	117 (92.1)
II	15 (4.1)	9 (7.1)
III	1 (0.3)	1 (0.8)
IV	0 (0.0)	0 (0.0)
HIV diagnosis		
During current pregnancy	147 (43.4)	46 (40.4)
≤3 y before pregnancy	92 (27.1)	33 (29.0)
>3 y before pregnancy	100 (29.5)	35 (30.7)
CD4+ T-cell count, cells/μL	368 [273–499]	386 [280–533]
<200	51 (14.1)	17 (13.4)
200–350	116 (32.1)	36 (28.4)
>350	194 (53.7)	74 (58.3)
HIV viral load	15,400 [2560–66,300]	12,800 [1830–51,100]
Log_{10} HIV viral load	4.2 [3.4–4.8]	4.1 [3.3–4.7]
Anthropometric		
Weight, kg	56.7 [52.0–62.0]	56.0 [51.0–62.0]
Height, cm	161 [158–166]	162 [158–167]
<150	11 (3.0)	2 (1.6)
BMI, kg/m^2	21.5 [20.0–23.4]	21.1 [19.8–23.5]
<18.5	36 (9.9)	11 (8.7)
18.5 to <25.0	274 (75.3)	98 (77.8)
≥25.0	54 (14.8)	17 (13.5)
Maternal micronutrient status at enrollment[2]		
Hb, g/dL	11.0 [10.2–11.8]	11.1 [10.3–11.9]
Anemia[3]	114 (32.0)	34 (26.8)
Plasma vitamin B-12, pmol/L	—	289 [213–405]
<221.0	—	38 (30.2)
<148.0	—	9 (7.1)
Serum folate, nmol/L	—	11.3 [8.6–14.9]
<13.5	—	84 (66.1)
<6.8	—	17 (13.4)
Vitamin D [$25(OH)D_3$], ng/mL	—	25.0 [19.0–32.0]
<30.0	—	83 (65.4)
<20.0	—	33 (26.0)

[1]Values are median [IQR] or *n* (%). Hb, hemoglobin.
[2]Maternal postpartum micronutrient data is available in first 98 d following delivery: 14.0 (0, 84.0) days.
[3]Maternal anemia was defined based on trimester-specific WHO criteria (trimester 1: Hb <11.0 g/dL; trimester 2: Hb <10.5 g/dL; and trimester 3: Hb <11.0 g/dL).

TABLE 2 Participant characteristics after enrollment: maternal micronutrient status postpartum, obstetric outcomes, and infant outcomes[1]

Characteristics	Entire cohort ($n = 367$)	Micronutrient substudy ($n = 127$)
Obstetric outcomes		
Miscarriage (<20 wk)	1 (0.3)	0 (0)
Stillbirth (≥20 wk)	10 (2.7)	0 (0)
Neonatal death (<28 d)	10 (2.8)	5 (3.9)
Sex, male	193 (54.2)	71 (55.9)
Gestational age at birth, wk	39.0 [37.6–40.1]	39.1 [37.6–40.3]
<37	58 (16.3)	19 (15.0)
<34	13 (3.7)	5 (3.9)
Birth weight, g	2900 [2670–3240]	2900 [2700–3240]
2500	56 (16.4)	15 (12.2)
Preterm and low birth weight	24 (7.0)	8 (6.5)
SGA[2]	79 (23.1)	26 (21.1)
Maternal micronutrient status postpartum[3]		
Hb, g/dL (≤7 d)	12.1 [11.1–13.0]	11.9 [11.1–13.0]
Anemia[4]	72 (22.3)	25 (21.4)
Hb, g/dL (≤28 d)	12.1 [11.1–13.0]	12.0 [11.1–13.0]
Anemia[4]	77 (22.1)	26 (20.8)
Plasma vitamin B-12, pmol/L	—	413 [309–573]
<221.0	—	2 (3.4)
<148.0	—	0 (0)
Serum folate, nmol/L	—	10.0 [7.0–18.1]
<13.5	—	38 (63.3)
<6.8	—	10 (16.7)
Vitamin D [25(OH)D_3], ng/mL	—	25.1 [18.2–32.5]
<30.0	—	41 (68.3)
<20.0	—	17 (28.3)
Infant outcomes		
Length, cm	48 [46–50]	48 [46–50]
LAZ	−1.09 [−1.78 to −0.03]	−1.09 [−1.78 to −0.03]
LAZ < −2	73 (21.8)	25 (21.2)
WAZ	−0.74 [−1.32 to −0.03]	−0.70 [−1.29 to −0.08]
WAZ < −2	40 (11.7)	13 (10.6)
WLZ	−0.02 [−0.83 to 0.84]	−0.06 [−0.91 to 0.71]
WLZ < −2	21 (6.9)	7 (6.4)
Ponderal index, g/cm^3	0.027 [0.025–0.029]	0.027 [0.025–0.029]
Head circumference, cm	34 [33–35]	34 [33–35]
Infant micronutrient status[5]		
Hb (at birth), g/dL	16.0 [14.0–17.0]	15.5 [14.0–17.0]
Anemia[4]	6 (3.4)	2 (2.7)
Hb (≤7 d of age), g/dL	16.0 [14.0–17.0]	16.0 [14.0–17.0]
Anemia[4]	13 (4.4)	3 (2.7)
Plasma vitamin B-12, pmol/L	—	479 [364–614]
<221.0	—	0 (0)
<148.0	—	0 (0)
Serum folate, nmol/L	—	14.0 [10.4–19.6]
<13.5	—	16 (44.4)
<6.8	—	2 (5.6)
Vitamin D [25(OH)D_3], ng/mL	—	17.4 [11.4–24.4]
<30.0	—	29 (82.9)
<20.0	—	20 (57.1)
<10.0	—	7 (20.0)

[1]Values are median [IQR] or *n* (%). Hb, hemoglobin; LAZ, length-for-age *z* score; SGA, small for gestational age; WAZ, weight-for-age *z* score; WLZ, weight-for-length *z* score.
[2]SGA was defined as <10th percentile of gestational age, using sex-specific INTERGROWTH criteria (44).
[3]Maternal postpartum micronutrient data were available in the first 98 d after delivery (median: 14.0 d; IQR: 0–84.0 d).
[4]Maternal anemia was defined as Hb < 12.0 g/dL and infant anemia was defined as Hb < 11.0 g/dL based on WHO criteria.
[5]Infant Hb concentrations were evaluated in the first 7 d of life; micronutrient concentrations were the first measurement in the first 98 d of life. Median (IQR) infant age at first measurement was 14.5 d (14.0–84.0 d) for vitamin B-12, 47.5 d (14.0–84.0 d) for folate, and 79 d (14.0–84.0 d) for vitamin D.

TABLE 3 Associations of maternal Hb concentrations and anemia with obstetric and infant outcomes[1]

Outcomes	*n*	Maternal Hb		Maternal anemia[2]	
		β ± SE or RR (95% CI)	*P* value	*β* ± SE or RR (95% CI)	*P* value
Obstetric outcomes					
Gestational age at birth, wk	356	−0.16 ± 0.10	0.09	0.44 ± 0.24	0.07
Preterm (<37 wk)	356	1.13 (0.92, 1.38)	0.26	0.69 (0.39, 1.21)	0.20
Birth weight, g	342	3.1 ± 22.6	0.89	35.5 ± 57.7	0.54
Low birth weight (<2500 g)	342	0.89 (0.73, 1.10)	0.29	1.42 (0.86, 2.35)	0.18
Preterm and low birth weight	342	1.08 (0.77, 1.52)	0.66	0.94 (0.39, 2.26)	0.90
Small for gestational age[3]	342	0.76 (0.65, 0.90)	**0.001**	1.88 (1.28, 2.77)	**0.001**
Infant outcomes					
Length, cm	336	−0.05 ± 0.12	0.68	0.06 ± 0.31	0.84
LAZ	335	−0.01 ± 0.06	0.88	−0.06 ± 0.16	0.70
LAZ < −2	335	1.01 (0.85, 1.20)	0.93	1.11 (0.72, 1.72)	0.65
WAZ	341	0.03 ± 0.05	0.60	0.01 ± 0.13	0.95
WAZ < −2	341	0.89 (0.69, 1.15)	0.37	1.50 (0.82, 2.75)	0.19
WLZ	306	0.08 ± 0.06	0.22	0.02 ± 0.16	0.91
WLZ < −2	306	0.89 (0.62, 1.27)	0.52	0.96 (0.39, 2.36)	0.93
Ponderal index, g/cm^3	336	0.0001 ± 0.0002	0.42	0.0001 ± 0.0004	0.79
Head circumference, cm	337	−0.04 ± 0.08	0.65	0.23 ± 0.22	0.29

[1]Linear and binomial regression models were used to evaluate the associations of maternal Hb concentrations and anemia at enrollment with obstetric and infant outcomes. Models were adjusted for the antiretroviral treatment arm, gestational age, maternal age, BMI, and log CD4 T-cell counts at enrollment. Hb, hemoglobin; LAZ, length-for-age *z* score; WAZ, weight-for-age *z* score; WLZ, weight-for-length *z* score.
[2]Maternal anemia was defined based on trimester-specific WHO criteria (trimester 1: Hb <11.0 g/dL; trimester 2: Hb <10.5 g/dL; and trimester 3: Hb <11.0 g/dL).
[3]Small-for-gestational age was defined as <10th percentile of gestational age, using sex-specific INTERGROWTH criteria (44).

Maternal baseline micronutrient status and infant micronutrient status

Table 6 presents the associations between maternal micronutrient status at enrollment and infant Hb, vitamin B-12, folate, and vitamin D concentrations during follow-up. Maternal Hb or micronutrient biomarkers were not significantly associated with infant Hb concentrations ($P > 0.05$). However, higher maternal vitamin B-12 concentrations at enrollment significantly predicted higher infant vitamin B-12 concentrations ($\beta \pm$ SE: 0.27 ± 0.12, $P = 0.02$); similarly, higher maternal folate concentrations at baseline were significantly associated with higher infant folate concentrations ($\beta \pm$ SE: 0.37 ± 0.16, $P = 0.02$), in multivariate models adjusting for antiretroviral treatment arm, gestational age at enrollment, maternal age, BMI, and log CD4 T-cell counts. Maternal folate status at enrollment also significantly predicted infant vitamin D concentrations: higher maternal folate concentrations were associated with higher infant 25(OH)D concentrations ($\beta \pm$ SE: 8.00 ± 3.82, $P = 0.04$), whereas maternal folate insufficiency predicted lower infant 25(OH)D concentrations ($\beta \pm$ SE: −7.05 ± 3.35, $P = 0.04$) in multivariate analyses.

Maternal ART and obstetric and infant outcomes

Supplemental Table 1 presents the associations of maternal ART intervention with pregnancy and infant outcomes. There were no significant differences in risk of obstetric outcomes by maternal ART intervention arm. However, maternal efavirenz treatment was associated with significantly higher infant WLZs ($\beta \pm$ SE: 0.43 ± 0.14, $P = 0.003$), higher ponderal index ($\beta \pm$ SE: 0.001 ± 0.0004, $P = 0.007$), and lower vitamin D concentrations ($\beta \pm$ SE: −6.67 ± 2.87, $P = 0.02$) than lopinavir, in analyses adjusting for gestational age at enrollment.

Discussion

This is one of the first studies to examine the burden of anemia and micronutrient deficiencies and their associations with adverse obstetric and infant outcomes among pregnant WLHIV initiating cART. Findings in this cohort in rural Uganda demonstrate that the prevalence of maternal anemia and micronutrient deficiencies was high early in pregnancy and predicted maternal and infant micronutrient status during the postpartum period. Maternal anemia was associated with increased risk of SGA, and increased vitamin D concentrations were associated with lower risk of infant wasting. However, in contrast to a priori hypotheses, higher maternal baseline vitamin D and vitamin B-12 concentrations were also associated with increased risk of preterm birth and lower birth weight, respectively. Further, the high prevalence of maternal micronutrient deficiencies despite the provision of micronutrient supplements suggests the importance of screening and nutritional interventions among WLHIV before conception.

The high prevalence of anemia in this cohort (26.8%) is consistent with previous studies among pregnant WLHIV (2, 5). (It is worth noting that women with severe anemia were excluded from enrollment, such that this is an underestimate of the prevalence of anemia.) The prevalence of micronutrient deficiencies—including maternal folate (66.1%), vitamin B-12 (30.2%), and vitamin D (65.4%) insufficiency—was similarly high in this study as in other cohorts of pregnant women (5, 8, 15, 50). This is among the first studies to date to examine the burden of micronutrient deficiencies among pregnant WLHIV initiating cART—and to examine the burden of anemia and micronutrient deficiencies in HIV-exposed children born to WLHIV on cART. Findings among children demonstrated that the prevalence of anemia (2.7%) and vitamin B-12 insufficiency (0.0%) was low at birth. However, the high prevalence of folate insufficiency (44.4%) and vitamin D deficiency (57.1%)

TABLE 4 Associations of maternal vitamin B-12, folate, and vitamin D concentrations at enrollment with obstetric and infant outcomes[1]

Outcomes	n	Maternal vitamin B-12		Maternal folate		Maternal vitamin D	
		β ± SE or RR (95% CI)	P value	n	β ± SE or RR (95% CI)	P value	n	β ± SE or RR (95% CI)	P value
Obstetric outcomes									
Gestational age at birth, wk	126	−0.48 ± 0.39	0.22	127	0.65 ± 0.39	0.09	127	−0.02 ± 0.02	0.45
<37	126	2.57 (0.90, 7.37)	0.08	127	0.50 (0.19, 1.34)	0.17	127	1.05 (1.00, 1.11)	**0.04**
Birth weight, g	122	−190 ± 88	**0.03**	123	117 ± 90	0.19	123	4.1 ± 4.9	0.40
<2500	122	4.78 (1.35, 16.93)	**0.02**	123	1.23 (0.67, 2.27)	0.51	123	0.98 (0.92, 1.05)	0.54
Preterm and low birth weight	122	17.23 (1.26, 235.90)	**0.03**	123	0.69 (0.31, 1.50)	0.35	123	1.05 (0.95, 1.15)	0.36
Small for gestational age[2]	122	1.91 (0.86, 4.22)	0.11	123	0.98 (0.45, 2.10)	0.95	123	0.97 (0.93, 1.01)	0.06
Infant outcomes									
Length, cm	117	−1.17 ± 0.49	**0.02**	118	−0.003 ± 0.49	0.99	118	0.02 ± 0.03	0.50
LAZ	117	−0.59 ± 0.26	**0.02**	118	−0.03 ± 0.26	0.91	118	0.01 ± 0.01	0.55
LAZ < −2	117	2.27 (1.05, 4.93)	**0.04**	118	1.63 (0.91, 2.93)	0.10	118	0.97 (0.92, 1.01)	0.18
WAZ	122	−0.45 ± 0.20	**0.03**	123	0.22 ± 0.21	0.28	123	0.01 ± 0.01	0.41
WAZ < −2	122	6.39 (1.43, 28.54)	**0.02**	123	1.17 (0.61, 2.25)	0.64	123	0.99 (0.92, 1.06)	0.73
WLZ	108	0.19 ± 0.29	0.51	109	0.43 ± 0.26	0.11	109	−0.01 ± 0.02	0.65
WLZ < −2	108	0.15 (0.01, 2.34)	0.17	109	0.60 (0.21, 1.74)	0.35	109	0.94 (0.89, 0.997)	**0.04**
Ponderal index, g/cm^3	117	−0.0005 ± 0.0008	0.55	118	0.0010 ± 0.0007	0.17	118	<0.0001 ± <0.0001	0.94
Head circumference, cm	120	−0.68 ± 0.34	**0.04**	121	0.63 ± 0.35	0.07	121	0.002 ± 0.02	0.93

[1] Linear regression models were used to evaluate the associations between maternal micronutrient status and infant hemoglobin, vitamin B-12, folate, and vitamin D concentrations; maternal and infant vitamin B-12 and folate concentrations were ln transformed to achieve normality before analysis. Models were adjusted for the antiretroviral treatment arm, gestational age, maternal age, BMI, and log CD4 T-cell counts at enrollment. LAZ, length-for-age z score; WAZ, weight-for-age z score; WLZ, weight-for-length z score.
[2] Small-for-gestational age was defined as <10th percentile of gestational age, using sex-specific INTERGROWTH criteria (44).

and insufficiency (82.9%) in children early in life is disconcerting and warrants further investigation. Findings suggest that anemia and micronutrient deficiencies—particularly folate and vitamin D—are common and are therefore important public health concerns in pregnant WLHIV on cART and their children.

Maternal anemia at the first prenatal visit was associated with a 2-fold increase in the risk of SGA infants; women had a 24% lower risk of SGA per 1-g/dL increase in baseline maternal Hb. These findings are consistent with previous research: maternal anemia during pregnancy has been associated with adverse pregnancy and neonatal outcomes in both HIV-uninfected (51–55) and HIV-infected populations, including increased risk of fetal loss, stillbirth, preterm birth, SGA, and low birth weight (1, 2, 18, 56, 57). The high burden of anemia among pregnant WLHIV and its associations with increased risk of adverse pregnancy and neonatal outcomes highlight a need to evaluate and improve the hematological status of WLHIV before and during pregnancy.

The prevalence of folate insufficiency was high in both mothers and their children (Tables 1, 2). Maternal folate concentrations at baseline predicted maternal and infant folate concentrations during the postpartum period. However, there were no significant associations between maternal folate status at enrollment and risk of adverse obstetric outcomes. Associations between low maternal folate status and increased risk of adverse pregnancy outcomes have been previously noted in HIV-uninfected (58) and HIV-infected populations (8). Of note, although women in our study received high-dose folic acid supplementation daily during pregnancy, they also received cotrimoxazole (TS) for malaria prophylaxis, which interferes with folate metabolism. In malaria-endemic areas, higher-dose prenatal folic acid is currently recommended along with malaria medications (e.g., sulfadoxine/pyrimethamine), in presumably HIV-uninfected pregnant women (although there is minimal evidence about the effects of concurrent folic acid and cotrimoxazole on folate status in pregnant WLHIV). The evidence for the efficacy, safety, and appropriate dosage of folic acid in malaria-endemic areas is currently being examined in a Cochrane systematic review to inform WHO guidelines—with specific considerations for pregnant women.

Vitamin B-12 insufficiency was common in pregnancy (Table 1), and maternal vitamin B-12 concentrations at enrollment significantly predicted postpartum maternal and infant vitamin B-12 status. Previous studies have noted a high prevalence of vitamin B-12 insufficiency in pregnant WLHIV and a correlation between maternal and infant vitamin B-12 status (8). In contrast to our a priori hypotheses, however, in this study higher vitamin B-12 concentrations at enrollment were also associated with increased risk of low birth weight and infant underweight. Findings are also in contrast to some previous observational studies among HIV-infected (1, 8) and HIV-uninfected women (59). However, current evidence from observational studies regarding the specific role of vitamin B-12 during pregnancy and risk of low birth weight is contradictory (60), and there are limited data from pregnant WLHIV and their children. The evidence for the efficacy of vitamin B-12 on specific maternal, pregnancy, and child health outcomes is being evaluated in a forthcoming Cochrane systematic review (61).

The prevalence of vitamin D deficiency and insufficiency was high in both mothers and infants in this population (Tables 1, 2); this is consistent with other studies in HIV-infected populations (15) and HIV-infected pregnant women (7, 62). Higher maternal vitamin D con-

TABLE 5 Associations of maternal micronutrient status at enrollment with maternal Hb, vitamin B-12, folate, and vitamin D concentrations postpartum[1]

	Maternal Hb postpartum			Maternal vitamin B-12 postpartum			Maternal folate postpartum			Maternal vitamin D postpartum		
Maternal baseline	***n***	**$\beta \pm$ SE**	***P* value**	***n***	**$\beta \pm$ SE**	***P* value**	***n***	**$\beta \pm$ SE**	***P* value**	***n***	**$\beta \pm$ SE**	***P* value**
Efavirenz vs. lopinavir	323	−0.13 ± 0.16	0.42	59	−0.13 ± 0.10	0.20	60	−0.20 ± 0.13	0.13	60	−0.39 ± 0.10	**0.0001**
Hb, g/dL	323	0.51 ± 0.07	**<0.0001**	59	−0.03 ± 0.04	0.44	60	0.11 ± 0.05	**0.04**	60	0.01 ± 0.05	0.91
Anemia[2]	323	−1.07 ± 0.17	**<0.0001**	59	0.12 ± 0.12	0.34	60	−0.20 ± 0.14	0.14	60	0.04 ± 0.12	0.76
Vitamin B-12, pmol/L	107	0.72 ± 0.31	**0.02**	29	0.48 ± 0.12	**<0.0001**	29	−0.45 ± 0.21	**0.03**	29	0.09 ± 0.12	0.44
<221.0	107	−1.04 ± 0.32	**0.001**	29	−0.33 ± 0.11	**0.004**	29	0.41 ± 0.18	**0.02**	29	−0.05 ± 0.11	0.67
Folate, nmol/L	108	−0.20 ± 0.32	0.53	30	−0.03 ± 0.14	0.80	30	0.56 ± 0.17	**0.0009**	30	0.14 ± 0.12	0.23
<13.5	108	0.16 ± 0.33	0.63	30	0.05 ± 0.14	0.75	30	−0.64 ± 0.17	**0.0002**	30	−0.10 ± 0.13	0.44
Vitamin D, ng/mL	108	0.27 ± 0.44	0.53	30	−0.25 ± 0.21	0.23	30	0.10 ± 0.31	0.75	30	0.10 ± 0.19	0.59
<30.0	108	−0.58 ± 0.32	0.07	30	0.01 ± 0.15	0.97	30	−0.03 ± 0.22	0.90	30	−0.11 ± 0.13	0.42
<20.0	108	−0.08 ± 0.35	0.83	30	0.18 ± 0.14	0.20	30	−0.10 ± 0.21	0.64	30	−0.12 ± 0.12	0.33

[1]Linear regression models were used to evaluate the associations between maternal Hb and micronutrient status at baseline and postpartum; vitamin B-12, vitamin D, and folate concentrations were ln transformed to achieve normality before analysis. Models were adjusted for the antiretroviral treatment arm, gestational age, maternal age, BMI, and log CD4 T-cell counts at enrollment. Postpartum micronutrient models were also adjusted for the days after delivery of the earliest measurement. Maternal Hb concentrations used the earliest measurement ≤7 d postpartum. Micronutrient concentrations postpartum used the earliest measurement ≤98 d. Hb, hemoglobin.

[2]Anemia was defined based on trimester-specific WHO criteria (trimester 1: Hb <11.0 g/dL; trimester 2: Hb <10.5 g/dL; and trimester 3: Hb <11.0 g/dL).

centrations were associated with significantly lower risk of wasting, consistent with previous studies among WLHIV noting associations between lower maternal vitamin D status and increased risk of stunting, underweight, and wasting in offspring (63). However, higher maternal vitamin D concentrations were also associated with an increased risk of preterm birth, which is in contrast to our a priori hypotheses. Although some previous studies noted an association between lower maternal vitamin D status and risk of preterm birth in pregnant WLHIV in Latin America (64) and the United States (65), findings from other observational studies have been divergent: no associations were noted between maternal vitamin D status and risk of preterm birth in other studies in ART-naïve pregnant WLHIV in Tanzania (66) or pregnant WLHIV on ART in Botswana (67). To that end, the efficacy of maternal vitamin D supplementation on specific obstetric and infant outcomes is currently being evaluated in a randomized trial among 2300 pregnant WLHIV initiating ART in Tanzania (68). The high prevalence of vitamin D deficiency in pregnant WLHIV and their infants in this study is disconcerting; however, its impact on risk of adverse obstetric and infant outcomes has not been established and warrants investigation.

Our study contributes novel data about the burden of anemia in pregnant WLHIV receiving cART, and nutritional status at birth in HIV-exposed, HIV-uninfected infants, a population with high risk of morbidity and mortality (69, 70). This is one of the few prospective studies to date to examine the burden and sequelae of micronutrient deficiencies in pregnant WLHIV and their infants (71, 72).

This study had several limitations. Prospective longitudinal data on micronutrient concentrations were only available for a subset of participants in the parent study. This limited our ability to examine changes in micronutrient status during pregnancy, and to examine temporal associations between maternal micronutrient concentrations and risk of obstetric and infant outcomes. This may constrain interpretation of the findings and their generalizability. Micronutrient concentrations assessed early in gestation may not reflect status periconceptionally, which may be critical for the development of pregnancy and infant outcomes. Although 1 of the inclusion criteria for the parent trial was 12–28 weeks of gestation, >96% of participants were in trimester 2 at enrollment; as a result, we were not able to examine findings by trimester (owing to the small *n* values in the trimester 1 and 3 strata). However, all analyses were adjusted for gestational age at enrollment, and findings were similar when analyses were restricted to women enrolled in their second trimester. Although this study was conducted within the context of a randomized controlled trial, the interpretation of observed associations is not causal. Future research is needed to examine mechanisms and determine the effects of micronutrients on longer-term maternal and child health outcomes.

In conclusion, in this prospective study of HIV-infected women initiating cART, anemia and micronutrient deficiencies were common. Maternal micronutrient status early in pregnancy significantly predicted maternal and infant micronutrient status postpartum. Lower Hb, folate, vitamin D, and vitamin B-12 concentrations were associated with increased risk of most adverse obstetric and infant outcomes. Given the lack of prospective data available on this vulnerable population, these findings are particularly concerning and underscore the need for further investigation. Further prospective studies are needed to inform the design of nutritional interventions to optimize the health of mothers living with HIV and their children.

TABLE 6 Associations of maternal micronutrient status at enrollment with infant Hb, vitamin B-12, folate, and vitamin D concentrations[1]

		Infant Hb		Infant vitamin B-12			Infant folate			Infant vitamin D		
Maternal baseline	***n***	***β* ± SE**	***P* value**	***n***	***β* ± SE**	***P* value**	***n***	***β* ± SE**	***P* value**	***n***	***β* ± SE**	***P* value**
Hb, g/dL	295	−0.03 ± 0.12	0.84	48	0.05 ± 0.05	0.25	48	−0.06 ± 0.06	0.31	47	−0.79 ± 1.28	0.54
Anemia[2]		−0.28 ± 0.33	0.38		−0.16 ± 0.11	0.15		0.04 ± 0.14	0.77		6.74 ± 3.01	**0.03**
Vitamin B-12, pmol/L	109	−0.37 ± 0.45	0.40	36	0.27 ± 0.12	**0.02**	35	0.17 ± 0.17	0.33	34	1.69 ± 3.79	0.66
<221.0		0.19 ± 0.47	0.69		−0.14 ± 0.11	0.22		0.01 ± 0.16	0.95		−0.21 ± 3.46	0.95
Folate, nmol/L	110	−0.52 ± 0.46	0.26	37	−0.07 ± 0.12	0.58	36	0.37 ± 0.16	**0.02**	35	8.00 ± 3.82	**0.04**
<13.5		0.16 ± 0.45	0.72		0.17 ± 0.11	0.13		−0.17 ± 0.16	0.30		−7.05 ± 3.35	**0.04**
Vitamin D, ng/mL	110	0.01 ± 0.02	0.69	37	−0.003 ± 0.007	0.70	36	0.003 ± 0.01	0.78	35	−0.06 ± 0.22	0.79
<30.0		−0.63 ± 0.45	0.17		−0.10 ± 0.14	0.47		0.02 ± 0.20	0.91		2.28 ± 4.04	0.57
<20.0		−0.15 ± 0.47	0.74		0.12 ± 0.12	0.29		0.15 ± 0.16	0.36		4.06 ± 3.46	0.24

[1]Linear regression models were used to evaluate the associations between maternal micronutrient status and infant Hb, vitamin B-12, folate, and vitamin D concentrations; maternal and infant vitamin B-12 and folate concentrations were ln transformed to achieve normality before analysis. Models were adjusted for the antiretroviral treatment arm, gestational age, maternal age, BMI, and log CD4 T-cell counts at enrollment. Infant Hb concentrations ≤7 d of life; infant vitamin B-12, folate, and vitamin D concentrations ≤98 d of life. Hb, hemoglobin.
[2]Anemia was defined based on trimester-specific WHO criteria (trimester 1: Hb <11.0 g/dL; trimester 2: Hb <10.5 g/dL; and trimester 3: Hb <11.0 g/dL).

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