View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Carolina Digital Repository

Plasma and breast-milk selenium in HIV-infected Malawian mothers are positively associated with infant selenium status but are not associated with maternal supplementation: results of the Breastfeeding, Antiretrovirals, and Nutrition study[1–3]

Valerie L Flax, Margaret E Bentley, Gerald F Combs Jr, Charles S Chasela, Dumbani Kayira, Gerald Tegha, Debbie Kamwendo, Eric J Daza, Ali Fokar, Athena P Kourtis, Denise J Jamieson, Charles M van der Horst, and Linda S Adair

ABSTRACT

Background: Selenium is found in soils and is essential for human antioxidant defense and immune function. In Malawi, low soil selenium and dietary intakes coupled with low plasma selenium concentrations in HIV infection could have negative consequences for the health of HIV-infected mothers and their exclusively breastfed infants.

Objective: We tested the effects of lipid-based nutrient supplements (LNS) that contained 1.3 times the Recommended Dietary Allowance of sodium selenite and antiretroviral drugs (ARV) on maternal plasma and breast-milk selenium concentrations.

Design: HIV-infected Malawian mothers in the Breastfeeding, Antiretrovirals, and Nutrition study were randomly assigned at delivery to receive: LNS, ARV, LNS and ARV, or a control. In a subsample of 526 mothers and their uninfected infants, we measured plasma and breast-milk selenium concentrations at 2 or 6 (depending on the availability of infant samples) and 24 wk postpartum.

Results: Overall, mean (±SD) maternal (range: 81.2 ± 20.4 to 86.2 ± 19.9 μg/L) and infant (55.6 ± 16.3 to 61.0 ± 15.4 μg/L) plasma selenium concentrations increased, whereas breast-milk selenium concentrations declined (14.3 ± 11.5 to 9.8 ± 7.3 μg/L) from 2 or 6 to 24 wk postpartum (all $P < 0.001$). Compared with the highest baseline selenium tertile, low and middle tertiles were positively associated with a change in maternal plasma or breast-milk selenium from 2 or 6 to 24 wk postpartum (both $P < 0.001$). With the use of linear regression, we showed that LNS that contained selenium and ARV were not associated with changes in maternal plasma and breast-milk selenium, but maternal selenium concentrations were positively associated with infant plasma selenium at 2 or 6 and 24 wk postpartum ($P < 0.001$) regardless of the study arm.

Conclusions: Selenite supplementation of HIV-infected Malawian women was not associated with a change in their plasma or breast-milk selenium concentrations. Future research should examine effects of more readily incorporated forms of selenium (ie, selenomethionine) in HIV-infected breastfeeding women. This trial was registered at clinicaltrials.gov as NCT00164736. *Am J Clin Nutr* 2014;99:950–6.

INTRODUCTION

The trace element selenium plays an important role in antioxidant defense, cell-mediated immunity, brain and thyroid function, and cardiovascular health (1). Overt selenium deficiency is rare (2). Symptoms in people with very low plasma selenium concentrations include cardiomyopathy, growth retardation, and a greater susceptibility to infection (3). Selenium concentrations tend to be low in HIV-infected individuals and decline as HIV progresses (4–7). It has been hypothesized that selenium becomes depleted in people with HIV because the body uses selenoproteins to suppress viral replication, or the virus uses selenium to create its own selenoenzymes (8), especially in the later stages of the disease (9). Low selenium concentrations have been associated with morbidity and mortality in HIV-infected populations (7, 10, 11). These effects can be limited by slowing the progression of HIV disease through the use of antiretroviral drugs (ARV)[4]. When

[1] From the Carolina Population Center (VLF, MEB, EJD, and LSA), Departments of Nutrition (VLF, MEB, and LSA) and Biostatistics (EJD), and Division of Infectious Diseases, School of Medicine (AF and CMvdH), University of North Carolina, Chapel Hill, NC; the USDA-Agriculture Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND (GFC); the University of North Carolina Project, Lilongwe, Malawi (CSC, D Kayira, GT, and D Kamwendo); the Division of Epidemiology and Biostatistics, School of Public Health, University of Witwatersrand, Parktown, South Africa (CSC); and the US CDC, Atlanta, GA (APK and DJJ).

[2] The Breastfeeding, Antiretrovirals, and Nutrition (BAN) study was supported by grants from the Prevention Research Centers 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), and the NIH Fogarty AIDS International Training and Research Program (DHHS/NIH/FIC 2-D43 TW001039 and R24TW007988; the American Recovery and Reinvestment Act). 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. Antiretrovirals used in the BAN study were donated by Abbott Laboratories, GlaxoSmithKline, Boehringer Ingelheim, Roche Pharmaceuticals, and Bristol-Myers Squibb.

[3] Address reprint requests and correspondence to VL Flax, Carolina Population Center, Campus Box #8120, Chapel Hill, NC 27516. E-mail: flax@unc.edu.

[4] Abbreviations used: ARV, antiretroviral drugs; BAN, Breastfeeding, Antiretrovirals, and Nutrition; LNS, lipid-based nutrient supplements.

Received August 16, 2013. Accepted for publication January 13, 2014.
First published online February 5, 2014; doi: 10.3945/ajcn.113.073833.

 © 2014 American Society for Nutrition

plasma selenium concentrations are generally sufficient, HIV-infected individuals who use ARV have maintained an adequate selenium status (12). This effect is likely a result of improved virologic control in individuals taking ARV. In settings in which selenium concentrations are already low at a population level, it may be necessary to combine both ARV and selenium supplementation to attain adequate selenium concentrations in HIV-infected individuals.

The selenium status varies worldwide and is largely dependent on food-selenium concentrations, which tend to reflect the selenium availability in soils (13). Malawi is among the countries with low amounts of selenium in soils and staple foods (14, 15). These low amounts have led to low dietary selenium intakes and low plasma selenium concentrations in Malawian adults (16, 17). HIV-infected Malawian women likely suffer from a double burden of low selenium intakes and increased selenium needs (18). This burden may be further exacerbated by the added micronutrient requirements of pregnancy and lactation (19).

In other countries, such as Finland and Poland, where soil selenium is low, the supplementation of lactating mothers with inorganic and organic selenium has increased maternal plasma and breast-milk concentrations as well as selenium intakes of their breastfed infants (20, 21). The effect of supplementation with inorganic selenium (in the form of sodium selenite) is generally smaller than that of organic selenium (in the form of selenomethionine) (22). The ability of oral selenium supplementation with inorganic or organic selenium to increase selenium concentrations in HIV-infected adults has also been shown (5, 9). In HIV-infected women, the effects of selenium supplementation on maternal and infant selenium status have not previously been reported to our knowledge.

The main aims of the current study were to measure the effects of selenium supplementation and ARV, which are used for the prevention of mother-to-child transmission, on maternal plasma and breast-milk selenium concentrations and to look at the subsequent intervention effects on infant plasma selenium concentrations. We hypothesized that women who received lipid-based nutrient supplements (LNS), which contained 127% of the Recommended Dietary Allowance for selenium during lactation, would have higher plasma and breast-milk selenium concentrations than those who were not supplemented, and maternal plasma selenium concentrations would be higher in mothers who received ARV than in mothers who did not receive ARV. Our secondary aims were to examine changes in maternal and infant selenium concentrations over time and to study the relation between maternal and infant selenium concentrations.

SUBEJCTS AND METHODS

Subjects and procedures

HIV-1–infected, pregnant women were recruited at antenatal clinics in Lilongwe, Malawi, for participation in the Breastfeeding, Antiretrovirals, and Nutrition (BAN) study from 2004 to 2009. Mother-infant pairs were eligible 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 concentration ≥70 g/L, and no previous ARV use (23).

With the use of a permuted-block method, mothers were randomly assigned by using a 2 × 3 factorial design to 1 of 6 study arms as shown in **Figure 1** (*see* reference 21 for details of the randomization procedure). Because this analysis examined intervention effects on the mother, we combined the infant ARV plus maternal LNS arm with the maternal LNS arm and the infant ARV arm with the control arm, which resulted in the following 4 groups that referred only to maternal interventions: LNS, ARV, LNS and ARV, and control. Assigned interventions began at delivery and continued through 28 wk. Mothers were counseled to exclusively breastfeed from 0 to 24 wk postpartum and wean their infants from 24 to 28 wk postpartum. LNS were designed to meet the energy, micronutrient, and protein needs of lactation. A daily 140-g dose of LNS contained 75 μg selenium in the form of sodium selenite; the complete nutrient content has been described elsewhere (24). Sodium selenite was selected for use in LNS rather than selenomethionine because the producer (Nutriset) uses only vitamins and minerals that are listed in the Codex Alimentarius and approved by the European Union, where the company is based. The maternal ARV intervention consisted of a highly active regimen, including zidovudine and lamuvidine as a single tablet (Combivir; GlaxoSmithKline) plus one or more other drugs (25). Mothers were initially given nevirapine as the third drug in their regimen. Nevirapine was discontinued in February 2005 and replaced with nelfinavir (Viracept; Roche). In January 2006, nelfinavir was replaced by lopinavir plus ritonavir (Kaletra; Abbott).

Blood and breast-milk samples were collected during regular study visits at 2, 6, and 24 wk postpartum. Plasma was separated from red blood cells within 60 min and kept at −70°C. Whole breast-milk samples were also frozen. Mothers were asked to report their adherence to LNS and ARV regimens at 1, 4, 8, and 21 wk. A questionnaire on socioeconomic characteristics was administered to mothers during screening.

To measure the effects of interventions on maternal and infant selenium concentrations, we selected a subsample who had breast-milk and maternal and infant plasma samples available at 2 time points, either at 2 and 24 wk (n = 358) or 6 and 24 wk (n = 168) postpartum. We originally planned to use samples from 2 and 24 wk postpartum to determine selenium concentrations as close to the beginning and end of supplementation as possible. We added 6 wk to the baseline group because some infants had inadequate quantities of stored plasma at 2 wk postpartum. We excluded multiple births and infants who were HIV positive. With the selected sample size, we have 83% power to detect an 8-μg/L mean difference in maternal plasma selenium concentrations between study arms.

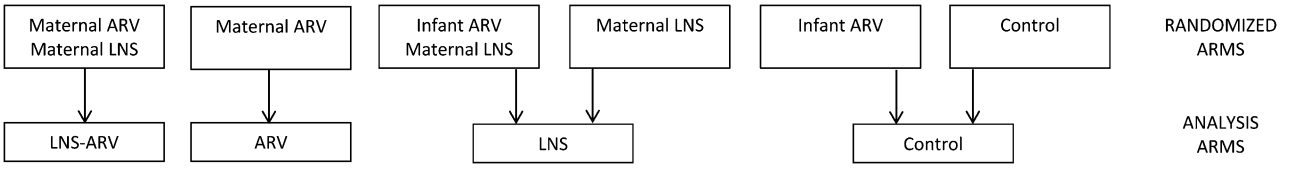

FIGURE 1. Randomly assigned Breastfeeding, Antiretrovirals, and Nutrition study arms and collapsed arms used in the analysis. ARV, antiretroviral drugs; LNS, lipid-based nutrient supplements; LNS-ARV, antiretroviral drugs and lipid-based nutrient supplements.

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 and the US CDC.

Laboratory analysis

Plasma and breast-milk selenium concentrations were measured at the USDA–Agriculture Research Service, Grand Forks Human Nutrition Research Center by using automated electrothermal atomic absorption spectrophotometry with a reduced palladium matrix modifier and an instrument equipped with L'Vov platforms and automated Zeaman-effect background correction (26). A daily calibration set for each batch was prepared by using certified standards (Alfa Aesar; Perkin Elmer). Calibration standards were analyzed at the beginning and end of each daily batch and at a 10% frequency within each batch. Plasma and breast-milk matrix effects were examined by using quantitative standards (National Institute of Standards and Technology) and spiked sample recovery tests. Laboratory tests of the LNS provided to mothers confirmed that the supplement contained the stated quantity (75 μg) of selenium.

Statistical analysis

We used a cutoff of the plasma selenium concentration at <80 μg/L to indicate a low selenium status in mothers. Adults with plasma selenium concentrations $\sim$80 μg/L appear to express major selenoproteins at maximal concentrations (27). We chose to report infant intakes of selenium from breast milk because there is no standard selenium adequacy for this age group. Infant selenium intake from breast milk was calculated by multiplying the mean breast-milk selenium concentration by the estimated breast-milk intake at each age (710 g/d at 2 or 6 wk postpartum and 780 g/d at 24 wk 24 wk postpartum) (28). The proportion with the recommended normative intake from the WHO/FAO (6 μg/d from 0 to 6 mo of age) (3) and adequate intake from the Institute of Medicine (15 μg/d from 0 to 6 mo of age) (19) was determined for each time point.

Our initial analysis tested intervention effects overall. We subdivided the sample into tertiles to determine whether interventions had a differential effect on the change in selenium by the baseline selenium concentration. The LNS used in this study contained selenite, which is not as bioavailable as organic selenium (ie, selenomethionine), and thus, we expected to see the strongest effects of supplementation in mothers with the lowest baseline concentrations. Means and proportions of background characteristics were compared between study arms by using a 2-factor ANOVA for continuous variables and logistic regression for categorical variables. Mean selenium concentrations from 2 or 6 to 24 wk postpartum were compared by using paired *t* tests. A 2-factor ANOVA was used to test effects of study interventions within tertiles. Multivariable linear regression models, with the timing of the early visit (2 or 6 wk postpartum) controlled for, were used to examine effects of BAN interventions and the baseline tertile on selenium concentrations in maternal plasma and breast milk. All analyses that examined effects of study interventions included LNS, ARV, and the interaction between LNS and ARV in the models. Multivariable models were also used to study associations between maternal and infant selenium concentrations. Statistical analyses were conducted with Stata 12.1 software (StataCorp).

RESULTS

Characteristics of mother-infant pairs generally did not differ by study arm, with the exception of the $CD4^+$ count at 24 wk postpartum (**Table 1**). As expected, women in study arms that received ARV had a higher median $CD4^+$ count at 24 wk postpartum. We also examined characteristics of mother-infant pairs in the selenium-analysis sample and those of other BAN participants. Mothers in the selenium subsample had a higher mean maternal age ($P < 0.01$) and lower mean BMI ($P < 0.05$), and a greater proportion had a $CD4^+$ count <250 cells/μL at

TABLE 1
Characteristics of mother-infant pairs in the selenium subsample by study arm[1]

	Control (n = 175)	LNS (n = 178)	ARV (n = 84)	LNS-ARV (n = 89)	P
Mothers					
Age (y)	26.5 ± 5.2[2]	26.5 ± 5.3	27.3 ± 5.3	26.3 ± 5.3	0.60
More than a primary education (%)	37	39	37	33	0.76
BMI at 2 wk postpartum (kg/m^2)	22.6 ± 2.6	22.8 ± 3.4	22.3 ± 3.3	22.3 ± 2.8	0.36
BMI $<$18.5 kg/m^2 at 2 wk postpartum (%)	3	5	6	8	0.33
$CD4^+$ at baseline (cells/μL)	421 (324, 570)[3]	447 (318, 591)	469 (304, 601)	400 (319, 584)	0.84
$CD4^+$ at 24 wk postpartum (cells/μL)	463 (319, 665)	491 (330, 698)	617 (439, 780)	607 (412, 796)	$<$0.001
$CD4^+$ $<$250 cells/μL at 24 wk postpartum (%)	10	12	3	4	0.02
No. of pregnancies	3.3 ± 1.7	3.3 ± 1.7	3.4 ± 1.5	3.4 ± 1.9	0.92
Infants					
Female (%)	47	53	43	45	0.34
Birth weight (kg)	3.0 ± 0.4	3.0 ± 0.4	3.1 ± 0.4	3.0 ± 0.4	0.61
Birth length (cm)	48.3 ± 1.8	48.1 ± 1.9	48.3 ± 2.0	48.2 ± 2.2	0.78

[1] $CD4^+$ counts at 24 wk postpartum included 481 participants. P values represent the overall significance for the difference in characteristics of study arms obtained from the 2-factor ANOVA for continuous variables and logistic regression for categorical variables. Models contained an interaction term for LNS-ARV to represent this study arm. ARV, antiretroviral drugs; LNS, lipid-based nutrient supplements; LNS-ARV, lipid-based nutrient supplements and antiretroviral drugs.

[2] Mean ± SD (all such values).

[3] Median; IQR in parentheses (all such values).

24 wk postpartum ($P < 0.01$), than did the rest of the study sample (*see* Supplemental Table 1 under "Supplemental data" in the online issue).

Maternal LNS and ARV adherence in the subsample was high. The proportion of mothers who reported eating the full daily dose on the previous day ranged from 87% to 96% at 1, 4, 8, and 21 wk postpartum, whereas the proportion of mothers who reported taking their ARV daily for the past 3 d ranged from 87% to 94% at the same time points.

We showed no differences in the mean change in selenium by study arm within any tertile (**Table 2**). In regression analyses, LNS that contained selenium and ARV were not associated with a change in the selenium concentration, whereas the trend across tertiles was positively associated with a change in maternal plasma or breast-milk selenium from 2 or 6 to 24 wk postpartum (*see* Supplemental Table 2 under "Supplemental data" in the online issue). Because there were no intervention effects on maternal selenium concentrations, we did not examine effects of interventions through breast milk on infant plasma concentrations.

Selenium concentrations varied widely at each time point (Table 2). Overall, mean maternal and infant plasma selenium concentrations increased (maternal: 81.2 ± 20.4 μg/L at 2 or 6 wk postpartum to 86.2 ± 19.9 μg/L at 24 wk postpartum; $P < 0.001$; infant: 55.6 ± 16.3 μg/L at 2 or 6 wk postpartum to 61.0 ± 15.4 μg/L at 24 wk postpartum; $P < 0.001$), whereas breast-milk selenium concentrations decreased (14.3 ± 11.5 μg/L at 2 or 6 wk postpartum to 9.8 ± 7.3 μg/L at 24 wk postpartum; $P < 0.001$) significantly over time. The direction of change in the selenium concentration differed by baseline tertile across all 3 outcomes, with selenium concentrations increasing in the low tertile and decreasing in the high tertile (Table 2).

On the basis of plasma selenium concentrations, we showed that 50% and 42% of mothers had a low selenium status (<80 μg/L) at 2 or 6 and 24 wk postpartum, respectively. Mean infant intakes of selenium from breast milk were estimated at 10.1 ± 8.2 μg/d at 2 or 6 wk postpartum and 7.7 ± 5.7 μg/d at 24 wk postpartum, indicating that 30% and 39% of infants did not achieve the FAO/WHO recommended selenium intake at 2 or 6 and 24 wk postpartum. According to the Institute of Medicine guidelines, 85% and 91% of infants did not achieve adequate selenium intakes from breast milk at 2 or 6 and 24 wk postpartum.

Maternal plasma and breast-milk selenium concentrations were strongly positively associated with infant plasma selenium concentrations at 2 or 6 and 24 wk postpartum (**Table 3**). The change in maternal plasma and breast-milk selenium from 2 or 6 to 24 wk postpartum was positively associated with the change in the infant selenium concentration (both $P < 0.001$) (not shown).

DISCUSSION

Despite good adherence to study interventions, we showed that LNS, ARV, and LNS and ARV did not affect maternal plasma or breast-milk selenium concentrations in the whole sample or in women with the lowest baseline selenium tertile. The form of selenium used in the supplement was likely one of the main factors that limited the effect of LNS on maternal selenium status. Inorganic sodium selenite is somewhat less readily absorbed than many food forms of selenium, particularly selenomethionine, which is the dominant one. More importantly, selenite can be incorporated only into selenoproteins. Twenty-five selenoproteins have been identified in humans, but many forms are present in only some tissues [eg, 9 forms are present in breast milk (29)]. In individuals with plasma selenium concentrations ≥80 μg/L, selenoproteins, such as glutathione peroxidase-3, are maximally expressed, and supplementation with selenite cannot be effectively incorporated (27). Consequently, selenite treatment produces small increases in concentrations of selenium in the plasma and breast milk of individuals who do not have low concentrations of the element. Unlike selenite, selenomethionine can also be incorporated nonspecifically into general proteins in lieu of methionine; for this reason, sources of selenomethionine produce greater increases in tissue selenium concentrations (20, 21, 29, 30). Good sources of selenomethionine include selenium-enriched yeast, which can be used in supplements, and high-protein foods produced on soils that are not deficient in selenium. When individuals are in a steady state concentration of selenium, the nonspecific component of plasma selenium represents a turning-over pool that releases selenium in the form of selenomethionine for incorporation into selenoproteins (26).

We expected that participants who received ARV would have had higher selenium concentrations because the virus would be suppressed and disease progression would be limited by the drugs (12). The lack of an ARV effect in the current study was likely explained by the high mean $CD4^+$ counts at 24 wk postpartum across study arms, even in subjects who did not receive drugs. Work by Rousseau et al (31) has indicated that there may be a threshold (eg, $CD4^+$ count <250 cells/mm^3) below which selenium concentrations begin to decline. This threshold was crossed by few women (~8%) included in this analysis.

The direction of change in selenium concentrations in postpartum women and infants has not been consistently established across studies. The decline in breast-milk selenium concentrations in our sample was similar to that in reports from the United States (32), Finland (20), Niger (33), and Burundi (34) but differed from that in studies in Belgium (35) and Japan (36), where there were no changes over time in the selenium concentration of mature human milk. Kumpulainen et al (20) have suggested that a gradual decline in breast-milk selenium concentration is physiologic even when maternal selenium consumption is sufficient. The increase in infant plasma selenium in this study was consistent with the results of other research, which indicated a small decline in selenium concentration from birth to 4 mo of age and an increase through the rest of infancy and to adulthood (37–39). The increase in maternal plasma selenium seen in this sample differed from the results in several other studies, in which maternal plasma selenium remained stable in nonsupplemented women postpartum (21, 32, 40), but was in agreement with results of studies in Niger (33) and Poland (41), where plasma selenium increased during lactation.

Mean maternal plasma and breast-milk selenium concentrations in our sample were within ranges reported in 2 reviews (2, 29). However, this result should not mask the fact that many of the mothers in this study had plasma selenium concentrations below those associated with maximal selenoprotein expression, which suggested the possibility of subclinical deficiency (42). The women also had substantially lower plasma selenium concentrations than HIV-infected women in Kenya (110 μg/L) and

TABLE 2
Selenium concentrations at 2 or 6 to 24 wk postpartum and the change in selenium over time by baseline tertile and study arm in a subsample of BAN study participants (n = 526)[1]

		Treatment arm														
		Control				LNS				ARV				LNS-ARV				
Outcome	Baseline tertile	n	2 or 6 wk postpartum	24 wk postpartum	Change in selenium	n	2 or 6 wk postpartum	24 wk postpartum	Change in selenium	n	2 or 6 wk postpartum	24 wk postpartum	Change in selenium	n	2 or 6 wk postpartum	24 wk postpartum	Change in selenium	P-change in selenium by arm[a]
	$\mu g/L$		$\mu g/L$	$\mu g/L$	$\mu g/L$		$\mu g/L$	$\mu g/L$	$\mu g/L$		$\mu g/L$	$\mu g/L$	$\mu g/L$		$\mu g/L$	$\mu g/L$	$\mu g/L$	
Maternal plasma selenium[b]																		
Low tertile	≤71	61	58.5 ± 9.0[2]	73.5 ± 15.2	15.0 ± 15.0	54	61.4 ± 9.0	78.1 ± 13.8	16.7 ± 15.4	31	59.6 ± 9.0	72.9 ± 16.2	13.3 ± 19.6	31	59.8 ± 11.1	76.8 ± 16.2	17.0 ± 18.8	0.77
Middle tertile	71.1–89	57	80.4 ± 5.5	82.4 ± 14.5	2.0 ± 16.1	60	80.3 ± 5.1	85.3 ± 16.7	5.0 ± 17.4	32	81.7 ± 5.4	86.6 ± 14.9	4.9 ± 14.6	31	79.5 ± 5.5	85.0 ± 13.7	5.5 ± 12.4	0.68
High tertile	>89	57	103.7 ± 10.3	100.3 ± 22.3	−3.4 ± 23.5	64	103.8 ± 14.9	96.5 ± 18.1	−7.3 ± 20.0	21	107.6 ± 14.0	103.8 ± 24.7	−3.8 ± 23.9	27	104.0 ± 11.8	100.9 ± 23.6	−3.1 ± 20.5	0.73
Breast-milk selenium[b]																		
Low tertile	≤9	61	4.5 ± 3.3	6.1 ± 5.3	1.6 ± 5.1	61	4.0 ± 3.3	7.2 ± 5.9	3.2 ± 6.4	25	4.6 ± 3.1	4.6 ± 4.8	0.0 ± 5.3	36	5.0 ± 3.5	6.3 ± 6.0	1.3 ± 6.2	0.86
Middle tertile	9.1–16	53	13.0 ± 1.0	11.2 ± 7.0	−1.8 ± 7.2	61	12.7 ± 2.2	9.3 ± 6.8	−3.4 ± 6.8	30	12.6 ± 1.7	10.5 ± 6.6	−2.1 ± 6.5	29	13.4 ± 2.0	8.8 ± 4.7	−4.6 ± 5.2	0.73
High tertile	>16	61	26.7 ± 11.6	14.1 ± 8.9	−12.6 ± 14.7	56	24.7 ± 11.3	11.9 ± 6.8	−12.8 ± 12.0	29	27.3 ± 13.2	12.8 ± 7.9	−14.5 ± 16.5	24	26.7 ± 13.8	15.9 ± 7.4	−10.8 ± 14.8	0.91
Infant plasma selenium[b]																		
Low tertile	≤50	55	40.1 ± 9.3	54.6 ± 12.8	14.5 ± 12.3	70	38.9 ± 10.8	55.1 ± 11.7	16.2 ± 16.8	28	35.3 ± 12.3	50.1 ± 14.6	14.8 ± 17.5	35	39.3 ± 9.6	51.3 ± 11.6	12.0 ± 13.7	0.73
Middle tertile	50.1–63	54	57.0 ± 3.9	60.4 ± 13.3	3.4 ± 13.1	60	56.5 ± 3.9	63.0 ± 15.5	6.5 ± 14.9	26	57.4 ± 3.8	61.9 ± 11.0	4.5 ± 11.5	31	57.1 ± 3.7	60.2 ± 14.5	3.1 ± 12.6	0.38
High tertile	>63	66	73.9 ± 7.5	71.0 ± 14.0	−2.9 ± 12.8	48	74.0 ± 7.7	64.2 ± 14.0	−9.8 ± 14.3	30	72.7 ± 74	70.5 ± 22.5	−2.2 ± 23.7	23	70.7 ± 6.3	71.2 ± 11.5	0.5 ± 10.9	0.60

[1] Models showed no significant effects of the study arms for maternal plasma selenium or breast-milk selenium (*see* Supplemental Table 2 under "Supplemental data" in the online issue). The effects of study arms were not tested for infant plasma because there were no effects in mothers, and infants were exclusively breastfed. Overall P values are reported because there were no significant effects of LNS, ARV, or the interaction between LNS and ARV on the change in selenium within each tertile. [a]Calculated by using a 2-factor ANOVA including LNS, ARV, and the interaction between LNS and ARV. [b]P-trend < 0.001 over tertiles by using multivariable linear regression models with the change in selenium as the outcome and controlling for the timing of the baseline measurement (2 or 6 wk postpartum). ARV, antiretroviral drugs; BAN, Breastfeeding, Antiretrovirals, and Nutrition; LNS, lipid-based nutrient supplements.

[2] Mean ± SD (all such values).

TABLE 3
Association of maternal plasma and breast-milk selenium concentration with infant plasma selenium concentration in a subsample of BAN study participants (n = 526)[1]

Outcome [infant plasma selenium (μg/L)]	β coefficient (95% CI)
Maternal plasma selenium	
2 or 6 wk postpartum	0.47 (0.42, 0.53)[a]
24 wk postpartum	0.41 (0.36, 0.47)[a]
Maternal breast-milk selenium	
2 or 6 wk postpartum	0.48 (0.37, 0.59)[a]
24 wk postpartum	0.57 (0.39, 0.74)[a]

[1] Values are from separate linear regression models that examined the association between maternal and infant plasma selenium or breast-milk and infant plasma selenium at baseline or the final measurement. [a]$P < 0.001$. BAN, Breastfeeding, Antiretrovirals, and Nutrition.

Tanzania (128 μg/L) (43, 44) but higher concentrations than women of unknown HIV status in rural Malawi (62 μg/L) (16). Breast-milk concentrations of selenium in our sample were low compared with many of the countries in a review by Dorea (29), including Niger (12.0 μg/L at 6 mo of age), Nigeria (24.2 μg/L at 3 mo of age), and Zaire (19.3 μg/L at 3 mo of age) but were similar to concentrations reported in Burundi (6.1 μg/L at 4 mo of age). As expected on the basis of low breast-milk selenium concentrations in mothers in the BAN study, a large proportion of infants in the current study did not achieve adequate selenium intakes, and estimated intakes at 24 wk postpartum were lower than in other countries where intakes from mature milk have been calculated (39). Infants are born with selenium reserves, but exclusively breastfed infants need the selenium in breast milk to maintain an adequate selenium status (29). Despite low selenium intakes from breast milk, selenium concentrations in BAN infants at 6 mo of age were higher than concentrations in a mixed group of HIV-exposed and unexposed infants in Zambia (49 μg/L at 6 mo of age) (45) and slightly lower than in infants of HIV-infected mothers in Tanzania (69 μg/L at 6 mo of age) (46). These concentrations indicated that at least a portion of infants in these countries have low selenium status and could benefit from higher selenium concentrations in maternal milk.

Three issues should be considered when interpreting these data. First, the generalizability of our findings may have been limited by criteria used to select the selenium subsample for this analysis. Although there were significant differences in a few characteristics of the selenium subsample compared with the rest of BAN participants, differences were small and generally not clinically important. Second, our baseline measurements were either at 2 or 6 wk postpartum, and values were somewhat different for these groups. However, both groups showed similar patterns of change for maternal plasma and breast milk, and relations between maternal and infant selenium concentrations did not differ by the timing of the baseline measurement. Third, we used published values for the breast-milk intake of exclusively breastfed infants to calculate adequate selenium intakes (28). These values were based on a small number of studies, and the lower age range covered the period from 0 to 2 mo of age. We suspect that breast-milk intakes at 2 wk postpartum may have been lower than the average intake during this period, and thus, we may have underestimated the proportion of infants who achieved adequate intakes at this time point.

In conclusion, intervention strategies to reduce the prevalence of a low selenium status in Malawian women's plasma and breast milk are needed. Recommendations to increase the intake of selenium-rich foods would be difficult in Malawi because the most common source of dietary selenium is maize, which is already eaten in large quantities and contains <7 μg Se/100 g (15, 17). Two other strategies that might be more feasible include the use of selenium-containing crop fertilizers or the use of organic selenium supplements. Fertilizers have proven to be effective in another country with low soil selenium (47), and they are widely used and frequently subsidized in Malawi. Alternatively, organic selenium supplements could be provided to women during antenatal visits and when mothers bring their infants for immunizations. Before choosing an intervention strategy, additional studies in HIV-infected mothers are needed to determine the dosage of selenomethionine in fertilizers or supplements that produces an optimal effect on plasma and milk concentrations.

We thank all the members of the BAN Study Team at the University of North Carolina at Chapel Hill, the CDC, Atlanta, and the University of North Carolina Project in Lilongwe. We are grateful to all women and infants who agreed to participate in the study. We also appreciate the laboratory work carried out by Laura Idso, Craig Lacher, and Setti Shahab-Ferdows.

The authors' responsibilities were as follows—MEB, CSC, DK, GT, DK, APK, DJJ, CMvdH, and LSA: designed and conducted the study; EJD and AF: were responsible for data management and provided statistical advice; GFC: led the laboratory analysis and contributed to the manuscript; VLF: carried out the analysis, drafted the manuscript, and had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript. Drug companies and nongovernmental entities that donated to or supported this study had no role in the design, implementation, analysis, or interpretation of the data. CMvdH received grant support from Abbott Laboratories and GlaxoSmithKline. VLF, MEB, GFC, CSC, DK, GT, DK, EJD, AF, APK, DJJ, and LSA reported no conflicts of interest.

REFERENCES

1. Rayman MP. Selenium and human health. Lancet 2012;379:1256–68.
2. Combs GF Jr. Selenium in global food systems. Br J Nutr 2001;85: 517–47.
3. Food and Agriculture Organization, World Health Organization. Human vitamin and mineral requirements: report of a joint FAO/WHO expert consultation Bangkok, Thailand. Rome, Italy: Food and Agriculture Organization, 2001.
4. Campa A, Baum MK. Micronutrients and HIV infection. HIV Ther 2010;4:437–69.
5. Cirelli A, Ciardi M, de Simone C, Sorice F, Giordano R, Ciaralli L, Constantini S. Serum selenium concentration and disease progress in patients with HIV infection. Clin Biochem 1991;24:211–4.
6. van Lettow M, Harries AD, Kumwenda JJ, Zijlstra EE, Clark TD, Taha TE, Semba RD. Micronutrient malnutrition and wasting in adults with pulmonary tuberculosis with and without HIV co-infection in Malawi. BMC Infect Dis 2004;4:61.
7. Kupka R, Msamanga GI, Spiegelman D, Morris S, Mugusi F, Hunter DJ, Fawzi WW. Selenium status is associated with accelerated HIV disease progression among HIV-1-infected pregnant women in Tanzania. J Nutr 2004;134:2556–60.
8. Hurwitz BE, Klaus JR, Llabre MM, Gonzalez A, Lawrence PJ, Maher KJ, Greeson JM, Baum MK, Shor-Posner G, Skyler JS, et al. Suppression of human immunodeficiency virus type 1 viral load with selenium supplementation: a randomized controlled trial. Arch Intern Med 2007;167:148–54.
9. Stone CA, Kawai K, Kupka R, Fawzi WW. Role of selenium in HIV infection. Nutr Rev 2010;68:671–81.
10. Baum MK, Shor-Posner G, Lai S, Zhang G, Lai H, Fletcher MA, Sauberlich H, Page JB. High risk of HIV-related mortality is associated

with selenium deficiency. J Acquir Immune Defic Syndr Hum Retrovirol 1997;15:370–4.
11. Kupka R, Mugusi F, Aboud S, Hertzmark E, Spiegelman D, Fawzi WW. Effect of selenium supplements on hemoglobin concentration and morbidity among HIV-1-infected Tanzanian women. Clin Infect Dis 2009;48:1475–8.
12. Jones CY, Tang AM, Forrester JE, Huang J, Hendricks KM, Knox TA, Spiegelman D, Semba RD, Woods MN. Micronutrient levels and HIV disease status in HIV-infected patients on highly active antiretroviral therapy in the Nutrition for Healthy Living cohort. J Acquir Immune Defic Syndr 2006;43:475–82.
13. Sillanpaa M, Jansson H. Status of cadmium, lead, cobalt and selenium in soils and plants of thirty countries. FAO Soils Bull 1992;65:1–195.
14. Donovan U, Gibson RS, Ferguson EL, Ounpuu S, Heywood P. The selenium content of staple foods from Malawi and Papua New Guinea. J Food Compost Anal 1991;4:329–36.
15. Chilimba AD, Young SD, Black CR, Rogerson KB, Ander EL, Watts MJ, Lammel J, Broadley MR. Maize grain and soil surveys reveal suboptimal dietary selenium intake is widespread in Malawi. Sci Rep 2011;1:72.
16. Gibson RS, Bailey KB, Ampong Romano AB, Thomson CD. Plasma selenium concentrations in pregnant women in two countries with contrasting soil selenium levels. J Trace Elem Med Biol 2011;25: 230–5.
17. Eick F, Maleta K, Govasmark E, Duttaroy AK, Bjune AG. Food intake of selenium and sulphur amino acids in tuberculosis patients and healthy adults in Malawi. Int J Tuberc Lung Dis 2009;13:1313–5.
18. World Health Organization. Nutrition counselling, care and support for HIV-infected women: guidelines on HIV-related care, treatment and support for HIV-infected women and their children in resource-constrained settings. Geneva, Switzerland: World Health Organization, 2004.
19. Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington, DC: National Academy Press, 2000.
20. Kumpulainen J, Salmenpera L, Siimes MA, Koivistoinen P, Perheentupa J. Selenium status of exclusively breast-fed infants as influenced by maternal organic or inorganic selenium supplementation. Am J Clin Nutr 1985;42:829–35.
21. Trafikowska U, Sobkowiak E, Butler JA, Whanger PD, Zachara BA. Organic and inorganic selenium supplementation to lactating mothers increase the blood and milk Se concentrations and Se intake by breast-fed infants. J Trace Elem Med Biol 1998;12:77–85.
22. Xia Y, Hill KE, Byrne DW, Xu J, Burk RF. Effectiveness of selenium supplements in a low-selenium area of China. Am J Clin Nutr 2005;81: 829–34.
23. van der Horst C, Chasela C, Ahmed Y, Hoffman I, Hosseinipour M, Knight R, Fiscus S, Hudgens M, Kazembe P, Bentley M, et al. Modifications of a large HIV prevention clinical trial to fit changing realities: a case study of the Breastfeeding, Antiretroviral, and Nutrition (BAN) protocol in Lilongwe, Malawi. Contemp Clin Trials 2009;30: 24–33.
24. Flax VL, Bentley ME, Chasela CS, Kayira D, Hudgens MG, Knight RJ, Soko A, Jamieson DJ, van der Horst CM, Adair LS. Use of lipid-based nutrient supplements by HIV-infected Malawian women during lactation has no effect on infant growth from 0-24 weeks. J Nutr 2012; 142:1350–6.
25. Chasela CS, Hudgens MG, Jamieson DJ, Kayira D, Hosseinipour MC, Kourtis AP, Martinson F, Tegha G, Knight RJ, Ahmed YI, et al. Maternal or infant antiretroviral drugs to reduce HIV-1 transmission. N Engl J Med 2010;362:2271–81.
26. Combs GF, Jackson MI, Watts JC, Johnson LK, Zeng H, Idso J, Schomburg L, Hoeg A, Hoefig CS, Chiang EC, et al. Differential responses to selenomethionine supplementation by sex and genotype in healthy adults. Br J Nutr 2012;107:1514–25.
27. Hill KE, Xia Y, Akesson B, Beoglin ME, Burk RF. Selenoprotein P concentration in plasma as an index of selenium status in selenium-deficient and selenium-supplemented Chinese subjects. J Nutr 1996; 126:138–45.
28. World Health Organization. Complementary feeding of young children in developing countries: a review of current scientific knowledge. Geneva, Switzerland: World Health Organization, 1998.
29. Dorea JG. Selenium and breast-feeding. Br J Nutr 2002;88:443–61.
30. Rayman MP. The use of high-selenium yeast to raise selenium status: how does it measure up? Br J Nutr 2004;92:557–73.
31. Rousseau MC, Molines C, Moreau J, Delmont J. Influence of highly active antiretroviral therapy on micronutrient profiles in HIV-infected patients. Ann Nutr Metab 2000;44:212–6.
32. Levander OA, Moser PB, Morris VC. Dietary selenium intake and selenium concentrations of plasma, erythrocytes, and breast milk in pregnant and postpartum lactating and nonlactating women. Am J Clin Nutr 1987;46:694–8.
33. Arnaud J, Prual A, Preziosi P, Favier A, Hercberg S. Selenium determination in human milk in Niger: influence of maternal status. J Trace Elem Electrolytes Health Dis 1993;7:199–204.
34. Benemariya H, Robberecht H, Deelstra H. Copper, zinc and selenium concentrations in milk from middle-class women in Burundi (Africa) throughout the first 10 months of lactation. Sci Total Environ 1995;164: 161–74.
35. Robberecht H, Roekens E, van Caillie-Bertrand M, Deelstra H, Clara R. Longitudinal study of the selenium content in human breast milk in Belgium. Acta Paediatr Scand 1985;74:254–8.
36. Higashi A, Tamari H, Kuroki Y, Matsuda I. Longitudinal changes in selenium content of breast milk. Acta Paediatr Scand 1983;72:433–6.
37. van Caillie-Bertrand M, Degenhart HJ, Fernandes J. Influence of age on the selenium status in Belgium and The Netherlands. Pediatr Res 1986;20:574–6.
38. Muntau AC, Streiter M, Kappler M, Roschinger W, Schmid I, Rehnert A, Schramel P, Roscher AA. Age-related reference values for serum selenium concentrations in infants and children. Clin Chem 2002;48: 555–60.
39. Valent F, Horvat M, Mazej D, Stibilj V, Barbone F. Maternal diet and selenium concentration in human milk from an Italian population. J Epidemiol 2011;21:285–92.
40. Mannan S, Picciano MF. Influence of maternal selenium status on human milk selenium concentration and glutathione peroxidase activity. Am J Clin Nutr 1987;46:95–100.
41. Wasowicz W, Gromadzinska J, Szram K, Rydzynski K, Cieslak J, Pietrzak Z. Selenium, zinc, and copper concentrations in the blood and milk of lactating women. Biol Trace Elem Res 2001;79:221–33.
42. Nève J. Human selenium supplementation as assessed by changes in blood selenium concentration and glutathione peroxidase activity. J Trace Elem Med Biol 1995;9:65–73.
43. Baeten JM, Mostad SB, Hughes MP, Overbaugh J, Bankson DD, Mandaliya K, Ndinya-Achola JO, Bwayo JJ, Kreiss JK. Selenium deficiency is associated with shedding of HIV-1–infected cells in the female genital tract. J Acquir Immune Defic Syndr 2001;26:360–4.
44. Kupka R, Msamanga GI, Xu C, Anderson D, Hunter D, Fawzi WW. Relationship between plasma selenium concentrations and lower gential tract levels of HIV-1 RNA and interleukin type 1β. Eur J Clin Nutr 2007;61:542–7.
45. Gibson RS, Kafwembe E, Mwanza S, Gosset L, Bailey KB, Mullen A, Baisley K, Filteau S. A micronutrient-fortified food enhances iron and selenium status of Zambian infants but has limited efficacy on zinc. J Nutr 2011;141:935–43.
46. Kupka R, Msamanga GI, Spiegelman D, Rifai N, Hunter DJ, Fawzi WW. Selenium levels in relation to morbidity and mortality among children born to HIV-infected mothers. Eur J Clin Nutr 2005;59:1250–8.
47. Aro A, Alfthan G, Varo P. Effects of supplementation of fertilizers on human selenium status in Finland. Analyst 1995;120:841–3.