Breast Milk Retinol Concentrations Are Not Associated with Systemic Inflammation among Breast-Feeding Women in Malawi[1]

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ABSTRACT The acute phase response and inflammation are associated with lower plasma retinol concentrations, but their effect on breast milk retinol concentrations is unclear. We measured plasma retinol concentrations, acute phase proteins, and breast milk retinol concentrations in 237 breast-feeding women at 2 wk postpartum in Blantyre, Malawi; 16.5% of the women had plasma retinol < 0.70 μmol/L and 14.8% had breast milk retinol < 1.05 μmol/L. Among women with and without inflammation [α_1-acid glycoprotein (AGP) > 1 g/L and/or C-reactive protein (CRP) > 5 mg/L], geometric mean (95% CI) plasma retinol was 0.89 (0.84, 0.94) and 1.05 (1.01, 1.17) μmol/L, respectively ($P < 0.0001$). Among women with and without inflammation, geometric mean (95% CI) breast milk retinol was 2.12 (1.89, 2.36) and 2.05 (1.75, 2.39) μmol/L, respectively ($P = 0.74$). In multiple linear regression models adjusting for age, parity, education, BMI, and days postpartum, plasma retinol concentrations were associated with plasma AGP and CRP concentrations ($P < 0.0001$ and $P = 0.01$, respectively), whereas breast milk retinol concentrations were unaffected by plasma AGP and CRP concentrations ($P = 0.22$ and $P = 0.86$, respectively). These findings suggest that breast milk retinol concentrations are not affected by systemic inflammation. J. Nutr. 135: 223–226, 2005.

KEY WORDS: • *acute phase response* • *inflammation* • *milk* • *retinol* • *vitamin A deficiency*

Vitamin A deficiency is a major cause of morbidity and mortality among children and women of childbearing age in developing countries worldwide (1). Vitamin A deficiency is characterized by immune suppression and increased susceptibility to infectious diseases (2). The identification of populations at risk for vitamin A deficiency is important for targeting groups that would benefit from interventions to improve vitamin A status. Vitamin A deficiency is defined to be of public health importance if ≥15% of a specific population has a plasma retinol concentration < 0.70 μmol/L (3).

Plasma retinol concentrations may be decreased during subclinical infections or inflammation because retinol bound with retinol-binding protein is a negative acute phase reactant (4). Acute phase proteins such as plasma α_1-acid glycoprotein (AGP)[3] and C-reactive protein (CRP) are markers for an acute phase response and can indicate the presence of a subclinical infection or inflammation (4,5). It was proposed that individuals with elevated acute phase proteins should be excluded from data analyses on surveys that rely on plasma retinol as an indicator of vitamin A status; thus, the prevalence of those with plasma retinol concentration < 0.70 μmol/L would be based only on those without markers of infection (5). Because subjects with vitamin A deficiency are more prone to infections (2,6,7), the exclusion of all individuals with biochemical indicators of infection could potentially underestimate the prevalence of vitamin A deficiency.

Breast milk retinol is considered a good indicator of vitamin A status in lactating women (8), but it is not well known whether breast milk retinol concentrations are affected by subclinical infection. A breast milk retinol concentration < 1.05 μmol/L is considered to be consistent with vitamin A deficiency in lactating women (8), and prevalence rates of <10%, ≥10 to <25%, and ≥25% of breast milk retinol <1.05 μmol/L are considered to indicate mild, moderate, and severe vitamin A deficiency, respectively, as a public health problem (1,3). Because much of the retinol in breast milk comes directly from the diet, we hypothesized that inflammation (9), as indicated by elevated acute phase proteins, would be associated with lower plasma retinol concentrations but not lower breast milk retinol concentrations. To address this hypothesis, we measured breast milk and plasma vitamin A concentrations and plasma acute phase proteins in breast-feeding women in Blantyre, Malawi.

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[3] Abbreviations used: AGP, α_1-acid glycoprotein; CRP, C-reactive protein; NIST, National Institute of Standards and Technology; OR, odds ratio; SRM, standard reference material.

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SUBJECTS AND METHODS

The study population consisted of women who gave birth at Queen Elizabeth Central Hospital in Blantyre, Malawi. Women were eligible for the study if they were HIV-negative, chose to breast-feed, resided in the Blantyre District, and were at least 18 y old. Written, informed consent was obtained for HIV screening, and pre- and post-test HIV counseling was provided. Women were enrolled in the study at 2 wk postpartum after giving written, informed consent. At the enrollment visit, demographic information was collected, a medical history, vital signs, physical exam, and anthropometry were conducted, and venous blood and breast milk were collected from the mothers. Maternal weight and height were measured to the nearest 0.1 kg and 1 cm, respectively, using a SECA 700 balance. The study protocol was approved by institutional review boards at Johns Hopkins School of Medicine in Baltimore, MD, and the College of Medicine, University of Malawi, Blantyre, Malawi.

Venous blood was collected from nonfasting women using trace element–free S-Monovette Li-Heparin syringes (Sarstedt Monovette); the blood was stored at 4°C until it was processed (600 × g, 7 min, room temperature). Plasma aliquots were made in sterile cryovials (Wheaton Science Products) and stored at −70°C. Casual milk samples (not controlling for the time since the last breastfeeding episode) were collected from mothers in the morning or early afternoon; ~5–7 mL of breast milk was collected from each breast by manual expression after sterilizing the nipple and areola with betadine solution. Whole breast milk aliquots were prepared by gentle swirling of the sample using a vortex mixer before pipetting the whole milk into 2-mL aliquots. Milk aliquots were frozen and stored at −70°C. A creamatocrit was not obtained, and milk vitamin A concentrations were expressed as retinol per volume of milk (μmol/L) rather than per gram of fat. Maternal HIV-1 infection was diagnosed on the basis of a positive rapid test (Determine 1/2 Rapid test, Abbott) and confirmed by a positive ELISA for HIV-1 antibodies (Wellcozyme, Wellcome Diagnostics). Women with positive HIV tests were excluded from this study.

Retinol was measured in plasma using HPLC and a modified method from the Nutrition Laboratory, Inorganic Toxicology and Nutrition Branch, Division of Laboratory Sciences, National Center of Environmental Health, Centers for Disease Control and Prevention (Rosemary Schleicer, personal communication). Briefly, tocol (Hoffmann-La Roche) was added to the samples as an internal standard, along with isopropanol to precipitate the proteins. Samples were extracted with hexane; extracts were dried under nitrogen and redissolved in 50:50 acetonitrile:ethanol. The mobile phase consisted of one pump in acetonitrile with 0.1% triethylamine, and a second pump in ethanol with 0.1% triethylamine. A gradient method was applied by varying the solvent concentrations from 85% acentonitrile:triethylamine to 50% aceonitrile:triethylamine and again to 85% acetonitrile:triethylamine.

Breast milk samples were randomly sampled from either the right or left breast milk aliquots in the repository, and retinol was measured in breast milk using HPLC. The saponification/extraction method was a slight modification of that of Tanumihardjo et al. (10). Briefly, samples were saponified in ethanol and KOH-HOH with gentle rotation for 1 h at 45°C, followed by 15 min on ice. Samples were extracted with hexane containing 0.1% BHT and dried under nitrogen. Once dried, the extracts were resuspended in 50/50 acetonitrile and ethanol. The HPLC method was the same as that used for plasma.

Standard curves were assayed periodically using standard reference material (SRM) 968C (National Institute of Standards and Technology; NIST), and sample retinol concentrations were calculated based on these curves. SRM 968C is a set of 2 plasma-based standards with NIST-certified levels of retinol. Quality control was assessed by repeated analysis of either a pooled human plasma or human breast milk control assayed at the beginning and end of each analysis. Between- and within-run CVs for controls were 18.1 and 10.6% for maternal plasma retinol, and 2.9 and 2.7% for breast milk retinol, respectively.

Plasma AGP was measured using a commercial radial immunodiffusion kit (Bindarid, The Binding Site). Plasma CRP was measured using a commercial ELISA (Virgo CRP 150, Hemagen Diagnostics). Controls provided by the Manufacturer were used as contols in both cases. Between- and within-run CVs for AGP and CRP were 1.4 and 1.4%, and 4.0, and 4.3%, respectively.

Plasma and breast milk retinol, AGP, and CRP were skewed to higher values and were transformed by natural log to achieve a normal distribution. Vitamin A deficiency in mothers was defined as plasma retinol concentrations < 0.70 μmol/L or breast milk retinol < 1.05 μmol/L (1). Elevated AGP was defined as AGP > 1 g/L and elevated CRP was defined as CRP > 5 mg/L (5,7). Systemic inflammation was defined as elevation in either or both AGP and CRP (AGP > 1 g/L and/or CRP > 5 mg/L). Student's t test and Wilcoxon rank-sum tests were used to compare continuous variables. χ^2 and exact tests were used to compare categorical variables between groups. Spearman correlations were used to examine the relation between plasma and milk retinol concentrations. A 1-sample t test for means was used to compare plasma retinol concentrations between all women and women without inflammation only. A 1-sample test for proportions was used to compare plasma retinol concentrations between all women and women without inflammation only. Multiple linear regression models were used to examine the relation between plasma or breast milk retinol concentrations and CRP and AGP concentrations, adjusting for age, parity, education, BMI, and days postpartum. Multivariate logistic regression models adjusting for age, parity, education, BMI and days postpartum were used to examine the relation between plasma or breast milk retinol concentrations and inflammation. All statistics were analyzed using SAS version 8.1. Differences were considered significant in this study at $P \leq 0.05$.

RESULTS

From February 2000 to April 2002, 250 women were enrolled in the study; of these, 237 (94.8%) had both plasma and breast milk samples available for laboratory analysis. Geometric mean (95% CI) breast milk retinol concentration was 2.09 (1.92, 2.29) μmol/L and geometric mean (95% CI) plasma retinol concentration was 0.93 (0.89, 0.97) μmol/L in the 237 women. There were 39 women (16.5%) who had plasma retinol < 0.70 μmol/L and 35 women (14.8%) who had breast milk retinol < 1.05 μmol/L.

The characteristics of women by inflammation status (inflammation defined as AGP > 1 g/L and /or CRP > 5 mg/L), are shown in **Table 1**. Women with inflammation had lower plasma retinol concentrations ($P < 0.0001$), and the proportion of women with plasma retinol < 0.70 μmol/L was higher in the women with inflammation compared with women without inflammation ($P = 0.05$). Breast milk retinol concentrations did not differ between women with and without inflammation ($P = 0.74$), and the proportion of women with breast milk retinol < 1.05 μmol/L also did not differ between the 2 groups ($P = 0.98$). The 2 groups did not differ by age, parity, education, or BMI. The Spearman correlation between maternal milk and plasma retinol concentrations at 2 wk postpartum was $r = 0.24$ ($P < 0.0002$).

If all women with elevated acute phase proteins were excluded from the analysis (5), the mean plasma retinol concentration increased from 0.93 to 1.05 μmol/L ($P < 0.001$). The proportion of women with plasma retinol concentrations < 0.70 μmol/L decreased from 16.5 to 9.4% when women with inflammation were removed from the analysis ($P = 0.10$) (5). If acute phase proteins were used to adjust plasma retinol (5), the proportion of women with plasma retinol concentrations < 0.70 μmol/L decreased from 16.5 to 10.1% ($P < 0.0001$). If all women with elevated acute phase proteins were excluded from the analysis (5), the geometric mean breast milk retinol concentration did not differ (2.09 vs. 2.05 μmol/L) ($P = 0.78$), nor did the proportion of women with breast milk retinol concentrations < 1.05 μmol/L (14.8 vs. 14.8%) ($P = 0.98$).

TABLE 1

Characteristics of lactating women in Malawi at 2 wk postpartum by inflammation[1,2]

Characteristic	No inflammation (n = 74)	Inflammation (n = 163)	P
Age,[3] *y*	22.5 (19.0, 28.0)	22.0 (20.0, 27.0)	0.62
Parity, *n*	2.53 (2.13,2.92)	2.65 (2.36,2.94)	0.71
Education, %			
None to Standard 8	50.00	61.73	0.09
Form 1 to Form 6	50.00	38.27	
BMI,[3,4] *kg/m²*	22.2 (20.8, 23.8)	22.7 (21.0, 25.1)	0.23
Plasma retinol, *μmol/L*	1.05 (1.01,1.17)	0.89 (0.84,0.94)	<0.0001
Plasma retinol < 0.70 *μmol/L, %*	9.46	19.63	0.05
Breast milk retinol, *μmol/L*	2.05 (1.75,2.39)	2.12 (1.89,2.36)	0.74
Breast milk retinol < 1.05 *μmol/L, %*	14.86	14.72	0.98

[1] Values are geometric mean (95% CI) or %, unless otherwise noted.
[2] Inflammation defined as AGP > 1 g/L and/or CRP > 5 mg/L.
[3] Median (lower and upper quartile).
[4] The BMI value was missing for 1 woman in the no inflammation group and 6 women in the inflammation group.

Multiple linear regression models were used to examine the association between plasma retinol concentrations (**Table 2**), breast milk retinol concentrations (**Table 3**), and AGP and CRP, adjusting for potential confounders. Of the 237 women with plasma and breast milk retinol concentrations, only 221 had all data available for the multiple linear regression analysis. After adjusting for age, education, parity, BMI, and days postpartum, AGP and CRP concentrations were associated with plasma retinol concentrations ($P < 0.0001$ and $P = 0.01$, respectively). After adjusting for age, parity, education, BMI, and days postpartum, AGP and CRP concentrations were not associated with breast milk retinol concentrations ($P = 0.22$ and $P = 0.86$, respectively). In addition, multivariate logistic regressions models adjusting for age, parity, education, BMI, and days postpartum showed that inflammation (AGP > 1 g/L and/or CRP > 5 mg/L) was associated with an increased risk of plasma retinol < 0.70 μmol/L [odds ratio (OR) 2.68, 95% CI 1.08, 6.64), but was not associated with breast milk retinol < 1.05 μmol/L (OR 1.15, 95% CI, 0.50, 2.63).

DISCUSSION

The present study suggests that breast milk retinol concentrations are relatively unaffected by systemic inflammation compared with plasma retinol concentrations. To our knowledge, this is the first study to examine the relation between plasma and breast milk retinol in the presence or absence of systemic inflammation among lactating women. Elevated acute phase proteins were closely associated with low plasma retinol concentrations, whereas no association was found between low breast milk retinol concentrations and elevated acute phase proteins. These findings suggest that, as an indicator of vitamin A status, breast milk retinol concentrations may be less susceptible to variation caused by subclinical infection and inflammation.

The 2 major sources of vitamin A in the circulation that are available for uptake by the mammary gland for secretion in breast milk are retinol carried by retinol-binding protein and retinyl esters carried by chylomicrons. Studies in animals suggest that chylomicrons provide at least one third to one half of vitamin A to mammary gland tissue during lactation for secretion into milk (11), and that in rats, milk retinol can vary with dietary vitamin A intake even when plasma retinol concentrations are relatively unaffected (12). Animal studies showed that there may be a homeostatic mechanism by which the transfer of retinol from the circulation to breast milk is protected within a fairly wide range of liver vitamin A stores and serum retinol concentrations (13). In rats, moderate restrictions of dietary fat or protein had little effect on the transfer of vitamin A from dam to pup, but more severe dietary restrictions had a negative effect on milk volume (13). All of these observations suggest that breast milk retinol is shielded from changes in plasma retinol because the mammary gland utilizes a large proportion of retinyl esters that have entered the circulation after absorption in the gastrointestinal tract.

Although it was proposed that individuals with biochemical markers for infection should be excluded from data analysis in surveys that rely upon plasma retinol concentrations (5), it

TABLE 2

Multivariate linear regression model for factors associated with plasma retinol concentrations

Characteristic	β (SE)	P-value
Log_{10}AGP	−0.322 (0.080)	<0.0001
Log_{10}CRP	−0.054 (0.021)	0.01
Age, *y*	0.005 (0.006)	0.39
Education[1]	−0.081 (0.044)	0.07
Parity	−0.008 (0.20)	0.69
BMI, *kg/m²*	0.008 (0.005)	0.12
Days postpartum	−0.024 (0.008)	0.002

[1] Education dichotomized as none to Standard 8, Form 1 to Form 6.

TABLE 3

Multivariate linear regression model for factors associated with breast milk retinol concentrations

Characteristic	β (SE)	P-value
Log_{10}AGP	−0.241 (0.198)	0.22
Log_{10}CRP	0.009 (0.051)	0.86
Age, *y*	0.014 (0.015)	0.37
Education[1]	0.134 (0.108)	0.22
Parity	−0.006 (0.049)	0.90
BMI, *kg/m²*	0.021 (0.013)	0.12
Days postpartum	−0.021 (0.019)	0.26

[1] Education dichotomized as none to Standard 8, Form 1 to Form 6.

appears that exclusion of individuals with subclinical infection could underestimate the prevalence of vitamin A deficiency. In the present study, the prevalence of vitamin A deficiency among breast-feeding women was 14.8%, based upon breast milk retinol concentrations, and 16.5%, based upon plasma retinol concentrations. If women with elevated acute phase proteins were excluded from the analysis, the prevalence of vitamin A deficiency as estimated using plasma retinol would be ~9% compared with a prevalence of 14.8% based upon the criterion of breast milk retinol. Although there was a trend toward fewer women with low plasma retinol when all women with inflammation were removed from the analysis, this decrease was of marginal significance, probably because there was a small number of women without inflammation who had low plasma retinol. When acute phase reactants were used to "adjust" the plasma retinol concentrations, the overall sample was larger and the "adjusted" prevalence of women with plasma retinol < 0.70 μmol/L was significantly lower. In this study population, the exclusion of individuals with elevated acute phase proteins or the use of "adjusted" retinol concentrations underestimated the prevalence of vitamin A deficiency by nearly 40%. Because vitamin A deficiency is a syndrome characterized by immune suppression, increased susceptibility to infections, and elevated acute phase proteins (6,7,14), the exclusion of individuals with elevated acute phase proteins or "adjustment" of plasma retinol concentrations may also lead to an underestimation of the prevalence of vitamin A deficiency in some populations.

One potential limitation of this study is that the fat content in breast milk samples was not determined, as a recent study suggested that the most accurate indicator of the response of breast milk retinol to vitamin A supplementation was the measurement of breast milk retinol per gram of fat in casual breast milk samples (15). Another study of breast-feeding women in Indonesia showed that breast milk vitamin A was most sensitive to changes in vitamin A status when expressed as retinol per volume of breast milk (16). In the present study, breast milk samples were collected at 2 wk postpartum; during this period, milk retinol concentrations tend to decline since time of delivery (17). Thus, it is possible that the prevalence of vitamin A deficiency may be underestimated when based on breast milk samples that were collected 2 wk postpartum. Because sample collection was not controlled for time since the last breast-feeding episode, the results are less precise than they might have been otherwise. It would be beneficial for future studies to examine breast milk retinol in relation to markers of inflammation at 1 mo postpartum or later when breast milk retinol concentrations have stabilized. It is not known whether the influence of the acute phase response differs during breast-feeding, and later analysis of breast milk retinol and inflammation could provide additional insight. The geometric mean breast milk retinol concentrations of ~2 μmol/L among lactating women in Malawi at 2 wk postpartum is similar to values reported in other populations in which vitamin A deficiency is prevalent. At 2 wk postpartum, breast milk retinol concentrations of 2.69 μmol/L were reported in Indonesia (18) and 1.41 μmol/L were reported in Bangladesh (15). A low-to-moderate correlation between plasma retinol and breast milk retinol concentrations was shown in previous studies, with correlations of 0.30 reported among Thai women who had been breast-feeding for >6 mo (19) and 0.32 among Indonesian women at 6 mo postpartum (8).

In summary, this study suggests that breast milk retinol concentrations are relatively unaffected by modest levels of systemic inflammation in this population of breast-feeding women in Malawi. In contrast, plasma retinol concentrations were lower in women who had elevated acute phase proteins. Exclusion or inclusion of women with elevated acute phase proteins may potentially underestimate or overestimate, respectively, the prevalence of vitamin A deficiency in a population in studies in which plasma retinol is used as the sole indicator of vitamin A status. If further studies in other populations corroborate these findings, this would suggest that breast milk retinol concentrations are a relatively good indicator of vitamin A status in breast-feeding women in whom subclinical infections may be common.

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