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Vitamin Content of Breast Milk From HIV-1–Infected Mothers Before and After Flash-Heat Treatment

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Abstract

Background—World Health Organization advocates heat treatment of expressed breastmilk (EBM) as one method to reduce postnatal transmission of human immunodeficiency virus (HIV) in developing countries. Flash-heat is a simple heat treatment method shown to inactivate cell-free HIV.

Objective—To determine the effect of flash-heat on vitamin content of milk.

Methods—Fresh EBM was collected from 50 HIV+ mothers in Durban, South Africa. Mothers washed their hands and then manually expressed 75–150 mL EBM into sterile jars. Milk was aliquoted to unheated controls or flash-heat (50 mL EBM in a glass jar heated in a 450-mL water jacket in an aluminum pan until water boiled, then EBM removed) simulating field conditions with an open flame. Samples were stored at -70°C and then analyzed for the effect of flash-heat on vitamins [A, ascorbic acid, riboflavin (B_2), pyridoxal-5-phosphate (B_6), folate, and B_{12}].

Results—Vitamin A was not significantly affected by flash-heat and vitamins B_{12} and C and folate increased significantly. Vitamins B_2 and B_6 were decreased to 59% (95% confidence interval 44 to 81) and 96% (95% confidence interval 92 to 99), respectively, of that found in unheated milk.

Conclusions—The percentage remaining after flash-heat suggests that most vitamin concentrations are retained after heating. Flash-heat may be a practical and nutritious infant feeding method for mothers in developing countries.

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Keywords

prevention of mother-to-child transmission; HIV; flash-heat; breastmilk; pasteurization; heat treatment; vitamins

INTRODUCTION

Globally, over 500,000 children were infected with human immunodeficiency virus (HIV) in 2006. Countries in sub-Saharan Africa bear the majority of the burden and are home to 90% of these infected children.¹ Although mother-to-child transmission of HIV can also occur via intrapartum and in utero exposure, approximately 40% of such infections are attributed to prolonged breast-feeding.² The risk of HIV transmission must be balanced with the benefits of breastmilk's protective immunological and nutritional properties. Infant formula, although recommended in developed countries to prevent mother-to-child transmission of HIV, has been shown to increase infant morbidity and mortality compared with breast-feeding in areas lacking safe water.³⁻⁶

Studies have shown exclusive breast-feeding for the first months of life to have a significantly lower risk of HIV transmission than does mixing breast-feeding with feeding other liquids or solids.⁷⁻⁹ Current World Health Organization recommendations state that HIV-positive mothers should breast-feed exclusively for 6 months unless replacement feeding is acceptable, feasible, affordable, sustainable, and safe, in which case avoidance of all breast-feeding is recommended.¹⁰ Cessation of breast-feeding should occur at 6 months only if a nutritionally adequate and safe diet is maintained.¹¹ Recent data, however, show this transition period from exclusive breast-feeding to complementary foods or replacement milk to be one of high risk for malnutrition and stunting,^{12,13} diarrhea and infant mortality,^{14,15} and increases in breastmilk viral load, suggesting that if a mother breast-feeds during this time, then the risk of HIV transmission could substantially increase.¹⁶ These findings illustrate that focusing primarily on exclusive breast-feeding for the first months of the infant's life is not enough to ensure positive infant health outcomes; safe and nutritious feeding options are needed during and after this transition period.

One potential option could be heat-treated breastmilk, listed by the World Health Organization as one modification of breast-feeding recommended in the context of HIV.¹⁷ We have previously shown that flash-heat, a simple high-temperature short time (HTST)^{18,19} home pasteurization method, could be an acceptable option²⁰ and is capable of inactivating cell-free HIV²¹ while maintaining the antimicrobial²² and immunological²³ properties unique to breastmilk. Flash-heat is similar to the commercial HTST methods, which heat to 72°C for 15 seconds and have been shown to effectively kill bacteria and cytomegalovirus yet have limited impact on vitamins, lactoferrin, and IgA. Thus, HTST is viewed as the preferable heating technique for human milk.^{18,24,25} Pilot data also suggest that nutritional properties are retained after flash-heat in breastmilk collected from healthy mothers in the United States.²⁶ Due to the vital nutritional role that breastmilk plays, particularly among infants in resource-poor areas, the objective of this study was to evaluate the effect of flash-heat on vitamin concentrations in breastmilk collected from HIV-positive mothers in South Africa.

MATERIALS AND METHODS

HIV-positive breast-feeding mothers, not currently receiving antiretrovirals or antibiotics, were recruited during postnatal clinic visits at an informal settlement in Durban, South Africa, between October 2004 and July 2005. As described elsewhere, 84 women agreed to participate in this study and after providing written, informed consent provided sociodemographic

information, a blood sample, and gave a total of 98 breastmilk samples, as some women donated additional breastmilk sample(s) on subsequent visits.²¹ Fifty breast-milk samples from 50 women were selected for vitamin analyses for this study based upon having sufficient breastmilk volumes obtained from manual expression and the availability of CD4⁺ T-lymphocyte assay results.

Participants were asked to wash their hands with soap and water and then manually express 75–150 mL of breastmilk into a sterile glass jar. Breastmilk samples were covered and stored immediately in an ice water bath and then transported within 2 hours to the laboratory where the same sterile glass jar was used for flash-heating. Fifty milliliters of each expressed breastmilk sample was aliquoted to be flash-heated and the remaining volume was aliquoted to be used as an unheated control.

The flash-heat method has been described in detail elsewhere.²⁶ Briefly, 50 mL of expressed breastmilk in an uncovered sterile 16-oz commercial glass food jar was placed in 450 mL of water in a 1:1 Hart brand 1-quart aluminum pan. Water and milk were heated together over a single-burner butane stove, used to imitate the intense heat of a fire, until the water reached 100°C and was at a rolling boil. The breastmilk was immediately removed from the water bath and allowed to cool to 37.0°C. Temperature data were collected at 15-second intervals using thermometer probes (Cole-Palmer Digi-Sense DualogR Thermocouple Thermometers). Similar to commercial HTST pasteurization methods,^{18,19} the temperature during flash-heat typically reached 72.9°C; temperatures were above 56.0°C for approximately 6 minutes 15 seconds.

Aliquots were stored frozen at –70°C and shipped frozen to the appropriate laboratories to await analyses. Vitamin A assays were performed at the Department of Nutrition, University of California, Davis, CA. Ascorbic acid (vitamin C), riboflavin (vitamin B₂), pyridoxal-5-phosphate (vitamin B₆), folate, and cobalamin (vitamin B₁₂) assays were performed at Mayo Medical Laboratories in Rochester, MN, and Wilmington, MA. Laboratory personnel were blinded to heat treatment. A spiked breastmilk sample was tested as an additional quality control during the study sample runs for ascorbic acid, thiamine, riboflavin, and pyridoxal-5-phosphate. Samples over the limit of detection were further diluted to get in the appropriate range for quantitation. For folate and B₁₂, standard quality control measures were performed during breastmilk analyses. Vitamin-specific assay methodologies are described below. Vitamin A: Samples were saponified by mixing with ethanolic potassium hydroxide containing pyrogallol and heated for 1 hour at 60°C. Retinol was extracted into hexane, using o-ethyl-retinal-oxime as an internal standard, and analyzed by reversed-phase high-performance liquid chromatography (HPLC). Ascorbic acid: An aliquot of sample was mixed with an equal volume of metaphosphoric acid, and after centrifugation, vitamin C was measured by HPLC. Riboflavin: After precipitation with metaphosphoric acid, riboflavin was measured by reversed-phase HPLC. Pyridoxal-5-phosphate: After protein precipitation with 10% metaphosphoric acid, pyridoxal-5-phosphate was measured using HPLC with on-line derivatization with sodium bisulfite and fluorometric detection. Folate and vitamin B₁₂: These were measured using automated, competitive chemiluminescent immunoassays on the Bayer ADVIA Centaur instrument. Thiamine was also assayed, but results were not interpretable and thus not included in the analysis.

Plasma CD4⁺ T-lymphocyte assays were performed at the Nelson Mandela School of Medicine, University of KwaZulu-Natal in Durban, South Africa, and breastmilk HIV assays by TaqMan Real-Time-RNA-PCR at the Viral and Rickettsial Disease Laboratory at the California Department of Health Services in Richmond, California.

The sample size estimate was based on the identified reference value in human milk for each vitamin to be studied.²⁷ Standard power calculations ($\alpha = 0.05$, $\beta = 0.8$) were performed to estimate the sample size required to detect a 25% decrease in concentration for each vitamin. Folate required the largest sample size ($n = 37$) to detect a minimum of 25% reduction, thus we selected a sample size of 50 for all vitamins analyzed. All 50 samples were included in the analysis of vitamin A, pyridoxal-5-phosphate, folate, and vitamin B₁₂, whereas 49 and 46 samples were included in analysis of ascorbic acid and riboflavin, respectively, due to interfering substances in several samples that did not allow accurate quantitation.

The effect of heating on vitamin concentrations was evaluated by estimating differences in medians and geometric means and their 95% confidence intervals using the paired Student *t* test on transformed data. To achieve normal distribution for analysis, we used the log transformation of vitamin A, folate, and riboflavin data and the square root transformation of vitamin B₁₂, ascorbic acid, and pyridoxal-5-phosphate data. Among the riboflavin assay results, 10 flash-heated samples and 1 unheated control sample had a value lower than the assay's limit of detection, <1.0 µg/L. These were investigated at lower (0.01 µg/L), midpoint (0.5 µg/L), and upper (0.99 µg/L) limits to determine worst and best case scenarios to determine the potential range of remaining vitamin concentrations. All statistical analyses were performed using Stata, version 8.0, Stata Corporation, College Station, Texas.

This study was approved by the Committees for the Protection of Human Subjects at the Universities of California at Berkeley and Davis and the University of KwaZulu-Natal.

RESULTS

Table 1 shows the maternal characteristics and socio-demographic data from the 50 HIV-positive mothers who provided breastmilk analyzed in this study. Compared with unheated control breastmilk samples, flash-heat treatment did not significantly affect levels of vitamin A ($P = 0.289$). We found significant decreases postheat in riboflavin and pyridoxal-5-phosphate concentrations to 59% and 96% of that found in the unheated milk controls ($P = 0.002$, $P = 0.008$, respectively) (Table 2; Fig. 1). Folate and vitamin B₁₂ concentrations increased significantly postheat ($P < 0.001$), whereas ascorbic acid increased with borderline significance ($P = 0.056$).

To quantify riboflavin levels with values <1.0 µg/L, we performed analyses using the lower limit of 0.01 µg/L, midpoint of 0.5 µg/L, and upper limit of 0.99 µg/L and found a corresponding 35.7% ($P = 0.007$), 59.4% ($P = 0.002$), and 65.0% ($P = 0.002$) remaining, respectively. We selected the midpoint of 0.5 µg/L as the imputed value for <1.0 µg/L.

DISCUSSION

In this study of breastmilk from HIV-positive mothers, we found that most vitamin concentrations were retained after flash-heat. The postheat increase in ascorbic acid, folate, and vitamin B₁₂ concentrations compared with unheated controls has been previously described^{18,26} and is hypothesized to be caused by heat-induced release of vitamins from binding proteins in the milk. With the exception of riboflavin, our findings are similar to previous reports of vitamin concentrations after Holder pasteurization of human milk, which showed that only pyridoxal-5-phosphate significantly decreased, whereas other vitamins remain stable²⁸; yet, that the vast majority of pyridoxal-5-phosphate was retained in our flash-heated samples is encouraging.

Riboflavin was most affected by the flash-heat process. These findings, however, should be interpreted with caution because we chose an imputed value of 0.5 µg/L for <1.0 µg/L, and the results could vary by as much as 25%. Previous studies have shown no statistically significant

decreases in riboflavin from Holder pasteurization (62.5°C for 30 minutes)²⁸ or from flash pasteurization (72.5°C for 15 seconds), an HTST method upon which the flash-heat method is based.¹⁸ Nevertheless, the effect of flash-heat on riboflavin should be investigated further.

These vitamins were selected for investigation because of their essential role in the maternal–infant dyad during lactation and thus considered priority micronutrients for lactating women and breastmilk quality and contribution to infant health.²⁹ Because infant stores of most micronutrients are limited, the role of breastmilk as a primary source of nutrition is vital, especially so in resource-poor areas where replacement milk may not be available or is limited as may be the quantity and quality of complementary foods. Vitamin A is important for growth and immune function and has been shown to maintain infant gut integrity and thus reduce infectious diseases.^{30–32} Although breastmilk vitamin A levels are not generally affected by maternal nutritional status, deficiencies are common in developing countries and have been associated with increased morbidity and mortality, especially during HIV infection.^{31,33,34} Ascorbic acid is necessary for collagen synthesis and thus growth and repair of tissue. Riboflavin is involved in vital metabolic processes and is necessary for normal cell function, growth, and energy production. Pyridoxal-5-phosphate is a coenzyme involved in metabolism of nerve tissue and is vital for DNA synthesis. Folate is an important cofactor essential for erythropoiesis and DNA synthesis, repair, and methylation. Vitamin B₁₂ plays a key role in pyrimidine and purine metabolism and together with folate regulates homocysteine levels.³⁵

The vitamin concentrations observed in the unheated breastmilk samples are in agreement with previous data comparing milk of well-nourished and of micronutrient-deficient women.³⁶ With the exception of folate, concentrations observed here are lower than previously reported in raw human milk from well-nourished women in industrialized countries.^{37–39} This is most likely because our study population included HIV-positive women from an underdeveloped semiurban area in South Africa, where approximately 80% of the mothers are unemployed and 50% of the homes have no running water, electricity, or sanitation. A previous study in a similar South African setting found that serum micronutrient deficiencies among both HIV-positive and HIV-negative mothers during lactation were common but that greater proportions of HIV-positive mothers had marginal vitamins A and B₁₂ status compared with those not infected.⁴⁰ Maternal vitamin deficiency during lactation, especially of water-soluble vitamins, is known to affect their concentration in breastmilk.²⁹

This study had several limitations. Use of an imputed value for riboflavin limits our ability to reach firm conclusions. We are also not able to comment on the potential bioavailability of the increased ascorbic acid, folate, and vitamin B₁₂ concentrations postheat after release from binding proteins. Additionally, although minerals are considered heat stable, this study did not investigate the effect of flash-heat on essential trace minerals required for infant growth and health, such as zinc which is known to reduce morbidity and mortality from diarrhea and pneumonia in children.⁴¹ Additional research is also needed to determine if variations in milk and water volumes, jar or pan size or shape, heat source, and even altitude could affect nutritional effects of flash-heat because this study investigated only one standardized heating protocol, 50 mL of breastmilk heated in a 450-mL water bath over a butane stove. The low vitamin concentrations we found in the unheated breastmilk samples, despite that 8% (4/44) of these mothers were taking vitamin supplements, may be related to maternal micronutrient deficiencies; however, maternal diet was not assessed, and plasma samples were not available to further evaluate maternal nutritional status.

In summary, the key finding of this study is that breastmilk treated by flash-heat retains the majority of its vitamin composition. Recent data have shown increases in diarrhea, gastroenteritis, malnutrition, and mortality with early cessation of breast-feeding in HIV-exposed infants receiving breastmilk substitutes.^{12–15,42,43} Flash-heated breastmilk could be

utilized as a “replacement milk” that is HIV free, safe, nutritious, affordable, available, and protective, especially during times of high risk, such as during mastitis or upon the addition of complementary foods. Multiple studies are currently under way in Africa to evaluate the feasibility of HIV-positive mothers flash-heating their breastmilk, with home-based support, after exclusive breast-feeding for 6 months. An efficacy trial will be needed in the future to determine the effect of flash-heated breastmilk on infant health outcomes.

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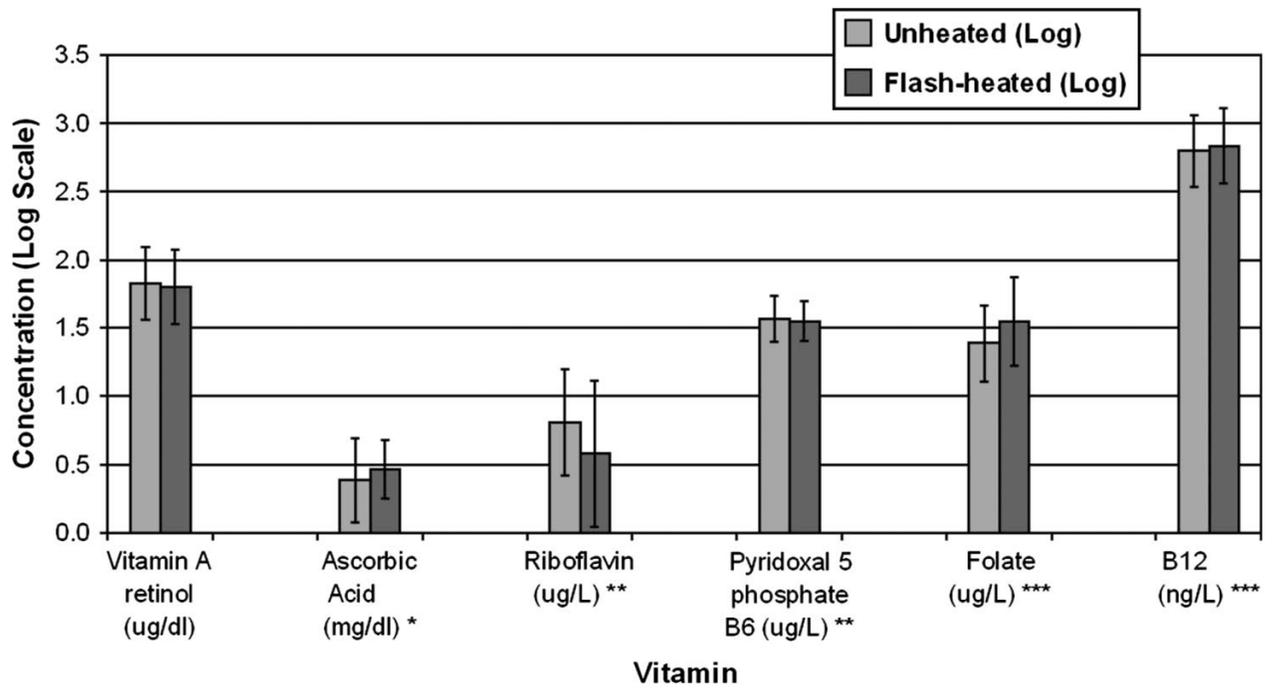


FIGURE 1.

Comparison of mean log (SD) of vitamin concentration in unheated vs flash-heated breastmilk (n = 50) (* $P < 0.05$ or borderline, ** $P < 0.01$, *** $P < 0.001$).

TABLE 1

Characteristics of Breastmilk Donors and Their Infants

Characteristic	Mothers	
	Mean (SD)	Range
Maternal age (yrs) (n = 50)	25.9 (4.9)	19.0–40.0
Maternal CD4+ count (cells/mm ³) (n = 50)	527.0 (254.6)	27.0–1173.0
Maternal HgB (g/dL) (n = 50)	11.5 (1.4)	6.7–14.4
Maternal weight (kg) (n = 50)	68.5 (11.9)	44.4–96.3
Maternal height (m) (n = 50)	1.6 (0.1)	1.4–1.7
Maternal BMI (kg/m ²) (n = 50)	27.5 (4.3)	20.0–37.5
Infant age (wks) (n = 50)	15.0 (11.0)	6.0–68.0
Infant birth weight (kg) (n = 48)	3.1 (0.4)	2.0–4.7
	n	%
Currently taking multivitamins		
Yes	4	8.0
No	44	88.0
Missing	2	4.0
Electricity		
Yes	11	22.0
No	19	38.0
Missing	20	40.0
Access to a refrigerator		
Yes	9	18.0
No	32	64.0
Missing	9	18.0
Piped water source		
Piped inside	14	28.0
Public tap	18	36.0
Piped in yard	12	24.0
Missing	6	12.0
Fuel for cooking		
Paraffin	32	64.0
Electricity	11	22.0
Gas	1	2.0
Missing	6	12.0

BMI, body mass index.

TABLE 2
Comparison of Vitamin Concentrations in Unheated and Flash-Heated Breastmilk From HIV-Positive Mothers

Vitamin	Unheated Median Concentration (Range)	Flash-Heated Median Concentration (Range)	Unheated Geometric Mean (SD)	Flash-Heated Geometric Mean (SD)	Geometric Mean Flash-Heated – Unheated (95% CI)	% of Concentration in Unheated Milk Detected Post-heat
Vitamin A retinol (µg/dL) (n = 50)	63.7 (14.9–313.0)	64.6 (15.0–256.6)	66.93 (1.84)	63.00 (1.88)	0.94 (0.84 to 1.05)	94.1
Ascorbic acid (mg/dL) (n = 49)*	2.7 (0.2–7.5)	3.0 (0.5–7.4)	2.43 (2.04)	2.93 (1.64)	1.21 (1.01 to 1.44)	120.6
Riboflavin (µg/L) (n = 46) [†]	6.0 (0.5–94.0)	4.0 (0.5–90.0)	6.41 (2.45)	3.81 (3.43)	0.59 (0.44 to 0.81)	59.4
Pyridoxal-5-phosphate B ₆ (µg/L) (n = 50) ^{*†}	36.5 (11.0–81.0)	34.0 (17.0–77.0)	36.94 (1.47)	35.47 (1.40)	0.96 (0.923 to 0.99)	96.0
Folate (µg/L) (n = 50) [‡]	19.45 (9.0–81.0)	45.6 (10.2–116.5)	24.41 (1.90)	35.11 (2.10)	1.44 (1.23 to 1.69)	143.8
Vitamin B ₁₂ (mg/L) (n = 50) [‡]	645.0 (124.0–1694.0)	770.0 (101.0–1920.0)	608.57 (1.82)	679.87 (1.89)	1.12 (1.06 to 1.18)	111.7

* Square root transformation used in analysis. All other calculations used log-transformed data.

[†] $P < 0.01$.

[‡] $P < 0.0001$.