Public Health Nutrition: 11(7), 747–750 doi:10.1017/S1368980008001857

Adequacy of vitamin A and fat in the breast milk of lactating women in south Sri Lanka

Chandrani Liyanage[1,*], Manjula Hettiarachchi[1], Prabhath Mangalajeewa[1] and Sarath Malawipathirana[2]

[1]Nuclear Medicine Unit, Faculty of Medicine, University of Ruhuna, PO Box 70, Galle, Sri Lanka:
[2]Chemical and Environmental Division, Industrial Technology Institute, Colombo, Sri Lanka

Submitted 24 May 2007: Accepted 5 January 2008: First published online 29 February 2008

Abstract

Objective: To determine vitamin A (retinol) and fat concentrations in breast milk during early lactation.

Methods: Healthy lactating women (*n* 88) aged between 18 and 35 years were randomly selected for the study from urban, semi-urban, rural and estate (plantation) sectors of Galle District. Their anthropometry was recorded; breast milk samples were collected from the right breast one hour after the last feed. Breast milk retinol was determined by HPLC and fat content by the crematocrit method.

Results: Subjects were in the 2nd to 9th month of lactation. Retinol concentrations of the breast milk samples ranged from 0·10 to 2·46 μmol/l, with a mean of 0·50 μmol/l, and correlated positively with parity (Pearson correlation coefficient, $r = 0{\cdot}307$; $P = 0{\cdot}01$) and negatively with period of lactation ($r = -0{\cdot}209$; $P = 0{\cdot}05$). The fat content of breast milk ranged between 5·09 and 56·46 g/l with a mean of 26·95 g/l. A significant difference in either breast milk fat or retinol content and mean birth weight of the babies was not seen between the groups. The ratio of retinol to fat in breast milk was positively correlated with weight ($r = 0{\cdot}274$; $P = 0{\cdot}01$) and height ($r = 0{\cdot}328$; $P < 0{\cdot}001$) of the mothers.

Conclusions: In this primary investigation on breast milk quality the fat content was found to be marginal; the majority of lactating mothers (92·0 %) were not providing the minimum daily requirement (1·05 μmol/l) of retinol to their babies.

Keywords
Breast milk fat and retinol
Lactating women
Period of lactation

Breast milk is a natural source of vitamin A and its close association with maternal vitamin A intake is well established[1]. Vitamin A-deficient lactating mothers may not have enough vitamin A in breast milk to maintain and build body reserves in their rapidly growing infants[2]. Vitamin A intake and status during the third trimester of pregnancy affect the retinol concentration in breast milk[3].

Vitamin A in breast milk is found almost exclusively in fat; thus factors that can affect breast milk fat concentration may affect the vitamin A concentration as well[4]. Fat is the macronutrient in milk that varies most in concentration and has been shown to be higher in well-nourished mothers than in their poorly nourished counterparts[5]. Fat concentration is higher in mature milk than in colostrum, and somewhat higher in breast milk of mothers from affluent compared with poor societies[6].

In Sri Lanka, no data are available on breast milk quality. The present study was therefore designed to assess the vitamin A (retinol) and fat concentrations of breast milk during 3–7 months of lactation and to determine any variation in the levels among women living in different geographical settings. We hypothesized that the economic and nutritional status of the subjects would be different in different settings of the study population and that the contents of vitamin A and fatty acids of breast milk would show differences according to the stratum to which the lactating mother belonged.

Sample and methods

Sample

The study took place in Galle District, Sri Lanka, between November 2004 and June 2005 with the approval of the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna. The sample of subjects was selected on the basis of a previous finding (0·55 μmol/l) of breast milk retinol concentration in lactating women[7] and to have 90 % power to detect an assumed difference of 0·1 % in breast milk retinol concentration. Accordingly, healthy lactating women (*n* 88) who were continuously breast-feeding, aged between 18 and 35 years, were included in the study after obtaining informed written consent. Height, weight, skinfold thickness (SFT) and mid upper-arm circumference (MUAC) of all mothers were

*Corresponding author: Email nmu_galle@yahoo.com

© The Authors 2008

https://doi.org/10.1017/S1368980008001857 Published online by Cambridge University Press

recorded, the past and present obstetrics history was obtained and a brief medical examination was conducted.

Breast milk samples were obtained from the right breast, which had not been used to feed the child during the previous hour, using a breast pump. The subjects were sufficiently instructed on how to use the breast pump (manufactured by Heinz Corporation) and they were given support during the milk collection by the investigators. Approximately 5 ml of milk was collected into dark brown glass bottles and transported to the laboratory in a cool box. After determination of fat content the remaining milk samples were frozen at −40°C until analysis for retinol, which was performed at the Industrial Technology Institute, Colombo, Sri Lanka.

Chemical analyses

Breast milk retinol

Breast milk retinol was analysed using the method developed by Tanumihardjo and Penniston[8] by HPLC after homogenizing and alkaline saponification to release the retinol from milk fatty acids. Internal standard used was 3,4-didehydroretinyl acetate (DRA; $C_{22}H_{30}O_2$; MW = 326). Breast milk samples (0·5 ml) were mixed with 40 μl DRA and suspended in 0·75 ml absolute ethanol; then 0·4 ml KOH–H_2O (50 : 50, w/v) was added followed by maintaining the mixture at 45°C for 1 h in a water bath and vortex mixing every 15 min for a period of 15 s. The sample was then extracted with 1 ml n-hexane and this was repeated twice. The total organic layer extracted was evaporated to dryness in an evaporator under N_2. Dried residue was re-suspended with 500 μl MeOH–CH_2Cl_2 mixture (50 : 50) and centrifuged prior to HPLC analysis.

Standard retinol solutions were prepared by mixing the retinol standard of highest purity (MW = 286·5, 97 %) with absolute ethanol and 3,5-bis-(t-butyl)-4-hydroxytoluene. The stock solution (500 μg/ml) was diluted with absolute ethanol to make a series of standards ranging from 1 to 10 μg/l. The HPLC analyses (Agilent series 1100 instrument; Agilent Technologies, Santa Clara, CA, USA) were carried out with MeOH–H_2O mixture (90 : 10, v/v) as mobile phase and separation was achieved with a C18 reverse phase column. Chromatograms for series of retinol standards were generated under identical HPLC conditions, and a calibration plot was constructed based on the peak area of each standard. The precise amount of retinol in each standard was determined on the basis of repeated measurements of absorbance of the concentrated standard in the calibration series at 325 nm. The calibration plot constructed was used for quantification of the retinol concentration in each individual sample.

Breast milk fat

The fat content of breast milk was determined using the crematocrit method[9]. Milk samples were drawn into capillary tubes, sealed and centrifuged at 5000 rpm for 5 min. Both the column of milk and the liquid fat that were clearly demarcated at the top of the sample were measured using a haematocrit reader to the nearest 0.5 mm. Each milk sample was centrifuged and measured three times. The crematocrit is the amount of liquid fat expressed as a percentage of the total milk sample in the tube to the nearest 0·5 %.

Statistical methods

The normality of data distribution was checked using the Kolmogorov–Smirnov test. Retinol concentrations in breast milk were not normally distributed and therefore were transformed logarithmically; they are reported as geometric mean with the 95 % confidence interval. Statistical analyses were performed using the SPSS for Windows statistical software package version 10·0 (SPSS Inc., Chicago, IL, USA). Correlation coefficients were calculated using Pearson correlation independently in each group. A value of $P < 0·05$ was considered significant.

Results

The baseline characteristics of the subjects according to their area of living are presented in Table 1. They were in the 2nd to 9th month of lactation with an average lactation period of 5·2 (SD 2·1) months. The estate (plantation sector) women had lower mean BMI compared with the other groups although the difference did not reach

Table 1 Anthropometry of the study subjects: healthy lactating Sri Lankan women aged 18–35 years

	Urban (*n* 26)		Semi-urban (*n* 22)		Rural (*n* 20)		Estate (*n* 20)		Total (*n* 88)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	30·4[a]	5·4	27·3[a]	5·5	25·2[a]	6·1	24·3[b]	5·9	27·1	6·1
Weight (kg)	50·45[a]	9·2	48·98[a]	8·6	49·23[a]	7·3	43·07[b]	6·2	48·13	8·4
Height (cm)	153·11[a]	5·6	153·05[a]	5·8	152·58[a]	5·5	149·64[a]	5·2	152·18	5·6
BMI (kg/m^2)	21·54[a]	3·8	21·00[a]	3·7	21·20[a]	3·2	19·17[a]	2·0	20·77	3·4
MUAC (cm)	24·03[a]	2·4	23·67[a]	2·4	24·44[a]	2·6	21·66[b]	1·4	23·49	2·4
SFT (mm)	15·80[a]	6·2	18·48[a]	5·0	17·65[a]	5·3	12·36[b]	3·6	16·11	5·6
Birth weight (kg)	2·84[a]	0·5	2·64[a]	0·5	2·83[a]	0·5	2·65[a]	0·4	2·74	0·5
Period of lactation (months)	5·42[a]	1·9	5·27[a]	2·4	5·95[a]	1·8	4·05[a]	2·2	5·19	2·1

MUAC, mid upper-arm circumference; SFT, skinfold thickness.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).

https://doi.org/10.1017/S1368980008001857 Published online by Cambridge University Press

Table 2 Breast milk retinol and fat concentrations of the study subjects: healthy lactating Sri Lankan women aged 18–35 years

		Retinol (μmol/l)		Fat (g/l)		Retinol:fat (μmol/g)	
	n	Mean	95 % CI	Mean	SD	Mean	SD
Area of living*							
Urban	26	0·53	0·41, 0·68	22·08	10·70	0·034	0·02
Semi-urban	22	0·34	0·27, 0·43	27·18	12·30	0·016	0·01
Rural	20	0·52	0·43, 0·63	27·89	14·00	0·026	0·02
Plantation (estate)	20	0·48	0·34, 0·68	33·76	11·50	0·020	0·01
Economic status (monthly income)†							
Rs. <5000	34	0·54	0·42, 0·69	31·68	12·04	0·023	0·02
Rs. 5000–10 000	41	0·42	0·35, 1·11	25·88	19·78	0·023	0·02
Rs. 10 000–20 000	5	0·56	0·33, 0·96	20·85	19·78	0·045	0·03
Rs. >20 000	8	0·44	0·29, 1·49	20·09	11·46	0·028	0·01
Period of lactation‡							
2–3 months	19	0·56	0·39, 0·79	31·54	10·99	0·025	0·02
4–6 months	47	0·49	0·42, 0·58	26·78	13·07	0·030	0·02
≥7 months	22	0·36	0·29, 0·46	24·55	12·62	0·021	0·02
Number of live births§							
≤2	73	0·45	0·39, 0·51	26·75	12·08	0·024	0·02
3 or 4	10	0·54	0·32, 0·92	29·29	15·54	0·030	0·02
≥5	5	0·71	0·36, 0·72	29·98	16·19	0·033	0·02

*Urban lactating mothers had significantly lower breast milk fat content ($P < 0·05$), and hence significantly higher retinol:fat ratio (ANOVA, $P = 0·03$), compared with the other area groups.
†Lactating mothers from families with income of Rs. 10 000–20 000 had significantly higher breast milk retinol:fat ratio (ANOVA, $P = 0·01$) compared with the other income groups.
‡Mothers lactating for ≥7 months had significantly lower breast milk retinol and fat contents (ANOVA, $P < 0·05$) compared with other lactation period groups.
§Lactating mothers with >3 live births (both 3 or 4 and ≥5) had significantly higher breast milk retinol content (ANOVA, $P < 0·05$) compared with mothers with ≤2 live births.

significance ($P = 0·09$). Furthermore, they were younger ($P < 0·05$) and had significantly lower mean weight ($P = 0·02$), SFT ($P < 0·001$) and MUAC ($P = 0·03$), showing a comparatively poorer nutritional status than women of the other groups. However, the mean birth weight of the babies was not significantly different between the groups.

Table 2 presents the concentrations of retinol and fat in breast milk, and the ratio of breast milk retinol to fat (μmol/g), according to area of living, economic status, period of lactation and parity. The retinol concentration ranged between 0·10 and 2·46 μmol/l, with a mean of 0.50 (95 % CI 0·41, 0·53) μmol/l, and was found to be correlated positively with parity (Pearson correlation, $r = 0·307$; $P = 0·01$) and negatively with period of lactation ($r = –0·209$; $P = 0·05$). The fat concentration ranged from 5·09 to 56·46 g/l with a mean of 26·95 (SD 12·5) g/l. The mean ratio of breast milk retinol to fat was 0·0249 (SD 0·018) μmol/g. This ratio was positively correlated with weight ($r = 0·274$; $P = 0·01$) and height ($r = 0·328$; $P < 0·001$) of the mothers.

Discussion

Retinol levels of breast milk were not correlated with economic status (a proxy for educational level) of the subjects. However, higher mean milk retinol concentrations, although not significant, were observed in urban and rural groups. This finding is in agreement with the comparatively higher mean birth weight (>2·8 kg) of their babies. Surprisingly, the fat concentration was significantly lower in the milk of women from the two highest income groups; the small sample size in each of these two groups may have been the reason for this difference. In contrast, the lowest income group had the highest mean milk fat concentration which was significantly different from the fat levels of other groups. Furthermore, mean milk fat concentration was observed to be significantly higher in estate women and significantly lower in urban women. The fat concentrations were not correlated with MUAC, SFT or the age of the lactating women. However, the estate women having a low mean age were found to have significantly higher mean milk fat concentration whereas urban women with a relatively higher mean age had a significantly low mean milk fat level. It was interesting to find that the retinol:fat ratio was positively correlated with weight and height of the subjects. This implies that the vitamin A and fat contents of breast milk are dependent on the general nutritional status of the mother. In making these interpretations, use of the crematocrit method and the variability in pumping duration could be considered as limitations.

The US Food and Nutrition Board has recommended that mature milk of well-nourished American mothers should contain 1·7 μmol vitamin A per litre[10] and an international standard of 1·05 μmol/l has been suggested for comparison. However, a study among Indonesian women reported that the breast milk vitamin A concentration was 0·60 (SD 0·29) μmol/l[11], which is slightly higher than the finding of the present study. It has been observed that parity appears to affect retinol levels in milk, showing a positive association; however, the association

https://doi.org/10.1017/S1368980008001857 Published online by Cambridge University Press

was found to be negative in Indonesian women[12]. The significant drop in retinol concentration with stage of lactation that is evident in the present study (Table 2) is in agreement with the findings of studies in Guatemala (1·40 μmol/l at 6 months decreasing to 0·33 μmol/l at 9 months)[5], the Philippines (1·26 μmol/l at 3 months, 0·88 μmol/l at 9 months)[13] and Ethiopia (1·16 μmol/l at 1·5–3·5 months, 0·74 μmol/l at 11·5–23·5 months)[14]. A drop in the fat concentration, although not significant, was also observed as lactation continued in the present study.

Despite the relatively lower levels of retinol in the milk of mothers from developing countries, breast milk is still considered the major source of vitamin A in the diets of their infants and young children. Thus it becomes important to improve maternal status to increase the vitamin A content of their milk, although Newman[13] reported in 1994 that vitamin A deficiency was rare among breast-fed infants even in parts of the world where the deficiency was endemic. Because the period of exclusive breast-feeding may be short and the number of breast feeds less in the case of working mothers, it becomes important to have a satisfactory level of breast milk retinol in order to develop good stores of vitamin A in the babies to meet their requirement during the weaning stage. However, lactating women suspected of having low stores and/or those unable or unwilling to increase their daily dietary intake of the vitamin would be expected to benefit from supplementation of vitamin A. It appears important to conduct a study with wider coverage to establish the fat and retinol concentrations of breast milk of lactating Sri Lankan women in order to implement intervention programmes with an aim of improving the vitamin A status of infants.

Acknowledgements

Contributors: M.H. was responsible for the research project and involved in all aspects; designing, sample selection, data collection, results and statistical analysis, and writing the paper. C.L. was responsible for the research project and involved in all aspects; designing, supervision of sample selection and data collection, results and statistical analysis, and writing and editing the paper. P.M. was responsible for the research project and involved in sample selection, data collection and paper writing. S.M. was involved in project design, was responsible for the analyses of breast milk retinol, and contributed to writing the paper. There are no conflicts of interest.

This investigation received financial support from the World Health Organization (SE/SRL NUT 001/RB04/EC 1.2.3). We acknowledge the technical assistance provided by Mr PH Nadun Suranga and Mr LA Sanath Dharmapriya of the Nuclear Medicine Unit, Faculty of Medicine, University of Ruhuna, Galle.

References

1. Brown KH, Akhter NA, Robertson AD & Ahmed MG (1986) Lactational capacity in marginally nourished mothers: relationship between maternal nutritional status and quantity of proximate composition of milk. *Pediatrics* **78**, 909–919.
2. Underwood BA (1994) Maternal vitamin A status and its importance in infancy and early childhood. *Am J Clin Nutr* **59**, 517S–524S.
3. Ortega RM, Andress P, Martinez RM & Sobalar AM (1997) Vitamin A status during the third trimester of pregnancy in Spanish women; influence on concentrations of vitamin A in breast milk. *Am J Clin Nutr* **66**, 564–568.
4. Stoltzfus RJ & Underwood BA. Breast milk vitamin A as an indicator of the vitamin A status of women and infants. *Bull World Health Organ* **73**, 703–711.
5. Ruel MT, Dewey KG, Martinez C, Flores R & Brown KH (1997) Validation in single daytime samples of human milk to estimate the 24-h concentration of lipids in urban Guatemalan mothers. *Am J Clin Nutr* **65**, 439–444.
6. Worthington-Roberts BS (1993) Human milk composition and infant growth and development. In *Nutrition in Pregnancy and Lactation*, 5th ed., pp. 347–401 [BS Worthington-Roberts and SR Williams, editors]. St Louis, MO: Mosby Year Book.
7. Butte NF & Calloway DH (1981) Evaluation of lactational performance of Navajo women. *Am J Clin Nutr* **34**, 2210–2215.
8. Tanumihardjo SA & Penniston KL (2002) Simplified methodology to determine breast milk retinol concentrations. *J Lipid Res* **43**, 350–355.
9. Lemons JA, Schreiner RI & Gresham EL (1980) Simple method for determining the caloric and fat content of human milk. *Pediatrics* **66**, 626–628.
10. Food and Nutrition Board (2001) *Dietary Reference Intakes*. Washington, DC: National Academy Press.
11. Muhilal, Permeisih D, Idjradinata YR, Muherdiyantiningsih & Karyadi D (1988) Vitamin A fortified monosodium glutamate and health, growth and survival of children: a controlled field trial. *Am J Clin Nutr* **48**, 1271–1276.
12. Stoltzfus RJ, Hakimi M & Miller KW (1993) High dose vitamin A supplementation for breast-feeding Indonesian mothers: effects on the vitamin A status of mother and infant. *J Nutr* **123**, 666–675.
13. Newman V (1994) Vitamin A and breast-feeding: a comparison of data from developed and developing countries. *Food Nutr Bull* **15**, 161.
14. Gebre-Medhin M, Vahlquist A & Hovander Y (1976) Breast milk composition in Ethiopian and Swedish mothers. 1. Vitamin A and β carotene. *Am J Clin Nutr* **29**, 441–445.

https://doi.org/10.1017/S1368980008001857 Published online by Cambridge University Press