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Evaluation of retinol levels in human colostrum in two samples collected at an interval of 24 hours

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Abstract

Objective: To evaluate retinol concentration in colostrum samples collected with a 24 hour interval.

Methods: Colostrum was collected from 24 recently-delivered mothers at two points in time, 0 hours (T0) and 24 hours later (T24), and a pooled sample of colostrum from T0 and T24 was also analyzed. Fat content was determined by creamatocrit, and retinol assayed by high performance liquid chromatography.

Results: When expressed in terms of volume of milk (µg/dL), retinol levels varied across T0, T24 and the pooled sample: 94.9±58.9, 129±78.6 and 111.9±60.4 µg/dL, respectively. However, when expressed with relation to fat content (µg/g), no significant difference was observed.

Conclusions: Retinol assayed in colostrum from a single sample should not be used as an indicator of vitamin A nutritional status, due to the great variation between samples collected at different times. It is suggested that results be expressed per gram of fat, in order to minimize variations resulting from the volume of milk.

J Pediatr (Rio J). 2007;83(4):377-380: Retinol, colostrum, fat.

Introduction

The composition of human milk varies over the first 2 weeks of lactation, with levels of certain nutrients becoming constant during the first month after delivery. The substance initially secreted is colostrum, which is produced for the first few days after delivery. From the fifth to the 21st day, what is secreted is known as transition milk, and the milk secreted thereafter is known as mature milk.[1,2] The nutritional composition of colostrum is particularly distinct, being rich in liposoluble vitamins.[3]

In addition to being influenced by the stage of lactation, the vitamin A contained in breastmilk may be altered by the fat content, as each feed progresses, and by factors specific to individual nursing mothers.[4-9]

Despite the great variation in retinol levels in colostrum, some authors have suggested that it could be used as an indicator of vitamin A nutritional status, since analyzing the nutrient in milk is non-invasive and access is easier.[10] The World Health Organization (WHO) recommends that, for retinol in milk to be representative, milk samples should be collected over a 24 hour period and assays should not be based on casual sampling, since retinol levels expressed per volume of milk may be sensitive to sampling errors.[11] In practice, however, collecting milk over 24 hours is not viable

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and so there is a need for practical alternative milk sampling procedures or other means of expressing retinol levels in order to reduce the variations currently observed.

The objective of this study was to evaluate the concentration of retinol in colostrum expressed per volume of milk and per gram of fat, based on two samples taken at an interval of 24 hours.

Methods

This study was approved by the Research Ethics Committee. The experiment was carried out during January and February of 2006 and recruited 24 healthy recently-delivered mothers selected at random from those who gave birth at Maternidade Escola Januário Cicco, Natal, RN, Brazil. They were aged from 18 to 40 years, had given birth to non-twin children at full term with no malformations and were enrolled within 12 hours of delivery. The sample size calculation was performed using the Statcalc module of Epi-Info 3.32. Assuming an average of 250 births per month and aiming at a confidence level of 95%, a minimum sample size of 25 subjects was estimated.

After free and informed consent had been obtained, a sample of colostrum was taken and split between two tubes, one of which was labelled "0 hours" and the other "pool." A second milk sample was taken 24 hours after the first. This was also divided in two with half stored in a tube labelled "24 hours" and the other added to the sample in the "pool" tube from the first collection.

The colostrum was collected by manual expression from a single breast that had not suckled previously. The first ejection was discarded in order to avoid fluctuations in the level of retinol. Sample volumes were from 1 to 3 mL and the milk was transported to the biochemistry laboratory on ice.

Fat content was determined by creamatocrit.[12] The remaining quantity of milk was then used for retinol extraction, using the method described by Giuliano et al.[13] modified as follows.

The total volume of milk collected was used for retinol extraction as a means of preventing the concentration of this vitamin from reducing, since, when an aliquot of milk is used for this analysis, a proportion of the retinol may be lost due to precipitation of fat globules which tend to come out of emulsion as time passes.[14] After the volume of milk collected had been measured, potassium hydroxide at 50% v/v (Vetec) and ethanol at 95% (Vetec) were added to the sample for the alkaline hydrolysis stage. The proportions of these reagents was as follows: for every 1 mL of milk, 2 mL of KOH at 50% and 1 mL of ethanol at 95% were added. The samples were then homogenized for 1 min and agitated in a water bath at 45 ºC for 2 h. The extraction reagent used was 2 mL of hexane (Merck), repeating the process three times. After each addition of hexane, samples were agitated for 1 min and centrifuged at 4,000 rpm for 10 min and the hexane layer separated into another tube.

A 3 mL aliquot from the hexane phase was evaporated in a nitrogen atmosphere using a water bath at 37 ºC. The resulting extracts were re-suspended in 1 mL of HPLC-grade methanol (Vetec).

The concentration of retinol in the samples was determined by HPLC in an LC-10 AD Shimadzu chromatograph, coupled to an SPD-10 A Shimadzu UV-VIS detector and a Chromatopac C-R6A Shimadzu integrator, with a 4.6 mm x 25 cm LC Shim-pack CLC-ODS (M) column. The chromatogram was developed under the following conditions: methanol mobile phase at 100% and flow rate of 1.0 mL/min.

The recovery test was performed by adding retinol acetate to the milk samples, resulting in more than 99% yield.

Identification and quantification of retinol from samples was by comparison with the retention time and area of a SIGMA all-trans retinol standard. The standard's concentration was confirmed by its specific extinction coefficient (e 1%, 1 cm = 1,750) in absolute ethanol (Vetec) and at a wavelength of 325 nm.

Concentrations of retinol in milk were expressed in µg/dL and also in µgROH/g of fat. This second figure was obtained by dividing the concentration of retinol per volume of milk (µg/dL) by the concentration of fat (g/dL). Values ≤ 30 µg/dL and ≤ 8 µgROH/g of fat are considered indicative of low retinol concentration in milk.[11]

Differences between means were compared using Student's *t* test for dependent samples. Differences were considered significant at $p < 0.05$.

Results

When expressed in terms of retinol per volume of milk (µg/dL), concentrations varied ($p < 0.05$) between the collection times (Table 1).

In contrast, when retinol concentrations were expressed as a function of the fat content of the milk, the level of retinol was stable, with values of 44.2±38.4, 46.8±38.3 and 43.0±31.9 µgROH/g of fat, for milk taken at 0 hours and 24 hours and pooled samples, respectively ($p > 0.05$).

No statistically significant differences were detected between fat concentrations (Table 1).

Discussion

The level of retinol in the colostrum from the women studied here is in line with values in published literature.[15]

Table 1 - Retinol and fat concentrations (mean ± standard deviation; n = 24) in milk, by time of collection

Collection time	Retinol (µg/dL)	Retinol (µg/g of fat)	Fat (g/dL)
Milk 0 hours	94.9±58.9*	44.2±38.4	2.9±1.7
Milk 24 hours	129.0±78.6	46.8±38.3	3.5±2.3
Pooled milk	111.9±60.4†	43.0±31.9	3.2±1.5

* Significant difference between 0 hours and 24 hours. Student's *t* test ($p < 0.023$).
† Significant difference 0 hours and pooled sample. Student's *t* test ($p < 0.02$).

There was no deficiency of vitamin A in the breastmilk according to parameters established by the WHO.[2]

When retinol concentrations were expressed in terms of volume of milk, a large variation was observed between collection times, although the concentrations in pooled samples were closer to vitamin A levels described in literature[15] (Table 1).

There was no difference in the concentration of retinol when results were expressed per gram of fat. This method of expressing retinol concentration in milk is used with the aim of controlling for variations resulting from sampling and because of the liposolubility of retinol. In other words, the volume of milk secreted varies more as lactation progresses than does the level of fat, which may alter results.[11]

In contrast with retinol levels, in our study it was possible to observe that the level of fat remained constant irrespective of the time of collection, confirming that lipids are more stable in the volume of milk than vitamin A is, despite a large proportion of the vitamin being found within fat globules. This has prompted some authors to suggest that these components enter the colostrum via distinct mechanisms.[15]

The level of retinol in breastmilk has been used in some studies as an indicator of vitamin A nutritional status and to assess the impact of programs to giving mothers vitamin A supplements.[5,9,10]

The WHO[11] recommends that when it is not possible to collect all milk for 24 hours, then results must be expressed as retinol per gram of fat, since this type of approach is less subject to variations than when expressed per volume of milk. Our results confirm this fact.

Rice et al.[5] sampled milk casually and found that vitamin A expressed per gram of fat better reflected the maternal response to vitamin A supplementation than did values expressed per volume of milk and recommended that the lipid content of milk samples should always be analyzed.

The results found here are a warning that retinol in colostrum from one-off samples should not be used as an indicator of vitamin A nutritional status, due to the great variability of the nutrient over different sample times. In order to minimize this problem, it is suggested that pooled samples be used or that results be expressed per gram of fat, in addition, for example, to associating retinol assays in milk with other types of analysis, such as serum retinol and dietary recall methods.

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References

1. Breastfeeding and the use of human milk. American Academy of Pediatrics. Work Group on Breastfeeding. Pediatrics. 1997;100:1035-9.
2. Euclydes MP. Nutrição do lactente: base científica para uma alimentação adequada. 2 ed. Viçosa: Metha; 2000.
3. Schweigert FJ, Bathe K, Chen F, Buscher U, Dudenhausen JW. Effect of the stage of lactation in humans on carotenoid levels in milk, blood plasma and plasma lipoprotein fractions. Eur J Nutr. 2004;43:39-44.
4. Haskell MJ, Brown KH. Maternal vitamin A nutriture and vitamin A content of human milk. J Mammary Gland Biol Neoplasia. 1999;4:243-57.

5. Rice AL, Stoltzfus RJ, de Francisco A, Kjolhede CL. Evaluation of serum retinol, the modified-relative-dose-response ratio, and breast-milk vitamin A as indicators of response to postpartum maternal vitamin A supplementation. Am J Clin Nutr. 2000;71:799-806.

6. Nascimento MBR, Issler H. Breastfeeding: making the difference in the development, health and nutrition of term and preterm newborns. Rev Hosp Clin Fac Med. 2003;58:49-60.

7. Ribeiro KDS, Dimenstein R. Níveis de retinol no leite materno ao início e final da mamada. Rev Panam Salud Publica. 2004;16:19-22.

8. Melo ILP, Ribeiro KDS, Dimenstein R. Estudo das variações dos níveis de retinol no colostro humano de parturientes a termo e pré-termo. Rev Bras Saude Mater Infant. 2004;4:249-52.

9. Meneses F, Trugo NMF. Retinol, β-carotene, and lutein+zeaxanthin in the milk of Brazilian nursing women: associations with plasma concentrations and influences of maternal characteristics. Nutr Res. 2005;25:443-51.

10. Stoltzfus RJ, Underwood BA. Breast-milk vitamin A as an indicator of the vitamin A status of women and infants. Bull World Health Organ. 1995;73:703-11.

11. World Health Organization, Nutrition Unit. Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes. (WHO/NUT/96.10). Geneva: WHO; 1996.

12. Lucas A, Gibbs JA, Lyster RL, Baum JD. Creamatocrit: simple clinical technique for estimating fat concentration and energy value of human milk. Br Med J. 1978;1:1018-20.

13. Giuliano AR, Neilson EM, Kelly BE, Canfield LM. Simultaneous quantitation and separation of carotenoids and retinol in human milk by high-performance liquid chromatography. Methods Enzymol. 1992;213:391-9.

14. Góes HC, Torres AG, Donangelo CM, Trugo NM. Nutrient composition of banked human milk in Brazil and influence of processing on zinc distribution in milk fractions. Nutrition. 2002;18:590-4.

15. Macias C, Schweigert FJ. Changes in the concentration on of carotenoids, vitamin A, alpha-tocopherol and total lipids in human milk throughout early lactation. Ann Nutr Metab. 2000;45:82-5.

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