Effect of maternal vitamin A supplementation on retinol concentration in colostrum

Evellyn C. Grilo a,*, Mayara S.R. Lima a, Lahyana R.F. Cunha b, Cristiane S.S. Gurgel a, Heleni A. Clemente a, Roberto Dimenstein a

a Universidade Federal do Rio Grande do Norte (UFRN), Natal, RN, Brazil
b Universidade Federal de Campina Grande (UFCG), Campina Grande, PB, Brazil

Received 7 February 2014; accepted 14 May 2014
Available online 2 September 2014

Abstract
Objective: To investigate the effect of vitamin A supplementation on the retinol concentration in colostrum under fasting and postprandial conditions.
Methods: This was a quasi-experimental study, with before and after assessments, conducted with 33 patients treated at a public maternity hospital. Blood and colostrum samples were collected under fasting conditions in the immediate postpartum period. A second colostrum collection occurred two hours after the first meal of the day, at which time a mega dose of 200,000 IU of retinyl palmitate was administered. On the following day, the colostrum was collected again under fasting and postprandial conditions. Serum and colostrum retinol concentrations were determined by high performance liquid chromatography.
Results: The serum retinol concentration was 37.3 (16.8-62.2) μg/dL, indicating adequate nutritional status. The colostrum retinol concentration before supplementation was 46.8 (29.7-158.9) μg/dL in fasting and 67.3 (31.1-148.7) μg/dL in postprandial condition (p < 0.05), showing an increase of 43.8%. After supplementation, the values were 89.5 (32.9-264.2) μg/dL and 102.7 (37.3-378.3) μg/dL in fasting and postprandial conditions, respectively (p < 0.05), representing an increase of 147%.
Conclusions: This study demonstrated that maternal supplementation with high doses of vitamin A in postpartum resulted in a significant increase of the retinol concentration in colostrum under fasting conditions, with an even greater increase after a meal.
© 2014 Sociedade Brasileira de Pediatria. Published by Elsevier Editora Ltda.
Este é um artigo Open Access sob a licença de CC BY-NC-ND
Introduction

Vitamin A is essential for human growth and development, preserves vision, and contributes to the proper functioning of the immune system, defending the body against infections.¹

Vitamin A deficiency (VAD) can lead to disorders such as xerophthalmia and night blindness in childhood, as well as anemia and low resistance to infections, which can increase the severity of infectious diseases and the risk of death. Children of preschool age and pregnant women are considered the populations at greatest risk for VAD; it is estimated that approximately one-third of the world’s population of preschoolers and 15% of pregnant women are biochemically deficient, mainly in Africa and Southeast Asia.²

Studies indicate that vitamin A deficiency constitutes a public health problem in the North and Northeast regions and some parts of Southeastern Brazil.³ In 2006, the National Demographic and Health Survey (NDHS) indicated that the prevalence of VAD in Brazil is 17.4% in children younger than 5 years and 12.3% in non-pregnant women of reproductive age.⁴

Pregnant and lactating women have a higher requirement of vitamin A, and the risk of deficiency is aggravated by low nutrient intake and the emergence of infections in these groups.¹ ³ The World Health Organization (WHO), the United Nations International Children’s Emergency Fund (UNICEF), and the International Vitamin A Consultative Group (IVACG) recommend the provision of high doses of vitamin A (200,000 IU) until the 60th day after delivery to postpartum women from regions where the deficiency of this nutrient is endemic.⁵

Breast milk is a source of energy and nutrients at adequate amounts for the infant’s nutrition, which includes proteins, lipids, carbohydrates, minerals, vitamins, lymphocytes, immunoglobulins, and growth factors.⁶ Colostrum is the milk secretion of the first days postpartum, and several studies have demonstrated the protective effect against neonatal mortality of feeding the newborn with this milk, especially when offered in the first hour of life.⁷

According to Black et al.⁸ the risk of a newborn to have its reserves exhausted is greater when there is maternal micronutrient deficiency. Thus, the vitamin A content of breast milk is the main determinant of the nutritional status of vitamin A in the newborn.

Ross et al.⁹ affirm that retinol is transferred to the milk in two ways: through the retinol binding protein (RBP) and through chylomicrons. However, the mechanism of vitamin A transfer to milk is yet to be fully understood in humans, and is being studied in animal models.¹⁰

Thus, the present study aimed to evaluate the effect of maternal vitamin A supplementation on retinol concentration in human colostrum under fasting and post-prandial conditions, aiming to contribute to the understanding of the mechanisms of retinol transfer to the mammary gland in humans, as most of the studies found in the literature were performed in animals.

Methods

This was a quasi-experimental intervention study, assessed before and after the intervention, carried out in a convenience sample. The study included 33 voluntary postpartum women aged 18 to 35 years treated at the obstetrics
department of a public maternity hospital. The sample size was calculated considering an alpha error of 5%, study power of 80%, and the size of the effect equal to 0.60. Based on these calculations, a minimum sample size of 33 participants was estimated.\textsuperscript{12}

Women with morbidities (diabetes mellitus, cancer, liver and gastrointestinal tract diseases, heart diseases, syphilis, and human immunodeficiency virus infection), adolescents, women who had preterm birth or with multiple or malformed fetuses, as well as those who reported having used supplements containing vitamin A during pregnancy or immediate postpartum period were not considered eligible for participation in the study. Participants who were in accordance with the selection criteria were included in the study after signing the informed consent form approved by the Ethics Committee in Research of the University Hospital.

Data and biological material collection

Blood (5 mL) and colostrum milk (2 mL) samples were collected from postpartum women under fasting condition, before the first meal of the day. Blood was obtained by venipuncture performed by a maternity nursing professional. The milk was collected by trained undergraduate students, through manual expression of a single breast, at the beginning and end of the breastfeeding. A second collection of colostrum occurred two hours after the first meal of the day (postprandial condition). Subsequently, a capsule of 200,000 IU (60 mg) of retinyl palmitate was administered. On the following day, a new colostrum milk collection was performed under fasting and postprandial conditions. The samples were collected in polypropylene tubes protected from light and kept under refrigeration during transport to the Food Biochemistry and Nutrition Laboratory. The blood samples were centrifuged for 10 minutes (500xg) for serum separation and removal, which was stored at -20°C as well as 500 μL aliquots of colostrum milk, until the analyses were performed. Maternal and obstetric data were collected from medical records of postpartum women.

Quantification of retinol in maternal serum and colostrum milk

Retinol was extracted from colostrum milk samples according to the adapted method of Giuliani et al.\textsuperscript{13} Extraction of serum retinol was performed according to the method of Ortega et al.\textsuperscript{14}

The dried extracts obtained from milk and serum samples were dissolved in absolute ethanol and 20 μL were analyzed in the high performance liquid chromatography (HPLC) equipment. The mobile phase used was 100% methanol in an isocratic system with a flow of 1.0 mL/min, and the wavelength used was 325 nm.

The identification and quantification of retinol in the samples was established by comparing the peak obtained in the chromatogram with the area of retinol standard (Sigma®, MO, United States). The concentration of the standard was confirmed by specific extinction coefficient for retinol (Ε 1%, 1 cm = 1780 to 325 nm) in absolute ethanol.\textsuperscript{15}

Method accuracy was evaluated by extraction recovery test. The mean recoveries obtained for the retinol acetate in milk and serum samples were 96% and 95%, respectively, suggesting extraction efficiency. Acceptable recovery intervals are generally between 80% and 110%.\textsuperscript{16}

The linearity of the curve was determined by assessing the proportionality between the detector response and different retinol standard concentrations. The calibration curve was obtained by linear regression (peak area as a function of standard concentration) and the obtained correlation coefficient was 0.990, allowing for the quantification of retinol by the external standard method.

Statistical analysis

The Shapiro-Wilks test was used for the assessment of normality of the retinol values. The study data consist of paired samples and presented a non-normal distribution, thus the nonparametric Wilcoxon test for paired samples was used. Differences were considered significant when p < 0.05. Spearman’s correlation test was used to evaluate the association between retinol levels in serum and colostrum milk. Statistical analyses were performed using the Minitab 15 Statistical software (Minitab, State College PA, United States) and R Development Core Team software (Lucent Technologies, NJ, United States).

Results

The median concentration of retinol in the serum of 33 women was 37.3 (16.8 to 62.2) μg/dL, considered adequate according to the reference values (> 20 μg/dL).\textsuperscript{17} In colostrum milk, the median retinol concentration before supplementation was 46.8 (29.7 to 158.9) μg/dL under fasting and 67.3 (31.1 to 148.7) μg/dL under postprandial condition (p < 0.05), showing an increase of 43.8% after the meal. After supplementation, the values were 89.5 (32.9 to 264.2) μg/dL and 102.7 (37.3 to 378.3) μg/dL under fasting and postprandial conditions, respectively (p < 0.05), equivalent to an increase of 14.7% (Table 1). Furthermore, comparison of retinol levels in colostrum milk under fasting condition before supplementation with postprandial retinol levels after supplementation showed an increase of 119.4%.

Retinol levels in the colostrum milk of fasting mothers before supplementation showed no correlation with their serum retinol values. The correlations between retinol levels of colostrum samples, collected at different times, are shown in Table 2.

Discussion

Until the end of pregnancy, a balanced diet is important to guarantee the transfer of nutrients to the fetus, in order to prepare it for birth and lactation. Vitamin A plays an important role during these periods, as it is involved in cell growth and proliferation processes, such as those that occur during pregnancy, lactation, and early childhood.\textsuperscript{9}

Maternal vitamin A supplementation in the immediate postpartum has been a widely used intervention in areas of high risk for vitamin A deficiency, and studies indicate that
Table 1  Retinol concentration (μg/dL) in colostr milk and serum of pregnant women in a public maternity hospital.

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>IQR 25-75</th>
<th>LL</th>
<th>UL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk (BS, fasting)</td>
<td>46.8</td>
<td>33.9 - 102.9</td>
<td>29.7</td>
<td>158.9</td>
</tr>
<tr>
<td>Milk (BS, postprandial)</td>
<td>67.3</td>
<td>44.1 - 105.5</td>
<td>31.1</td>
<td>148.7</td>
</tr>
<tr>
<td>Milk (AS, fasting)</td>
<td>89.5</td>
<td>63.1 - 137.7</td>
<td>32.9</td>
<td>264.2</td>
</tr>
<tr>
<td>Milk (AS, postprandial)</td>
<td>102.7</td>
<td>76.6 - 150.5</td>
<td>37.3</td>
<td>378.3</td>
</tr>
<tr>
<td>Maternal serum (BS, fasting)</td>
<td>37.3</td>
<td>31.2 - 50.3</td>
<td>16.8</td>
<td>62.2</td>
</tr>
</tbody>
</table>

IQR 25-75, interquartile interval; LL, lower limit; UL, upper limit; BS, before supplementation; AS, after supplementation.

This intervention results in an increase in retinol levels in breast milk.

Serum retinol levels observed in the present study (37.3 μg/dL) were lower than those found in women from Germany (49.3 μg/dL) and Southeastern Brazil (48.1 μg/dL), and similar to those observed in women from Northeastern Brazil (40.1 μg/dL).

Colostr milk retinol levels in fasting conditions before supplementation were lower than those found in women from Northeastern Brazil (100.3 μg/dL and 92.9 ± 50 mg/dL). These differences may be due to the larger sample size in the aforementioned studies and the fact that retinol levels in milk were expressed as mean and standard deviation, as they showed normal distribution.

In the present study, when retinol levels in milk under fasting and postprandial conditions were compared, a significant increase was observed in the latter on the two days of collection. In the statistical analysis, the values of r indicated a strong correlation (r = 0.65) between these levels, both before and after supplementation (Table 2).

Animal studies have demonstrated that vitamin A is transferred to breast milk from two sources: retinol-binding protein (RBP) and retinyl esters transported by lipoproteins (chylomicrons).

Under fasting conditions, almost all circulating retinol is associated with RBP, and levels of this protein do not vary with the ingestion of large amounts of vitamin A. However, retinyl esters transported by chylomicrons vary according to the content of vitamin A in the diet, and these lipoproteins contribute to the transport of retinol to the mammary gland.

In this study, the increase in Colostr retinol after a meal suggests that in humans, in addition to the mechanism of retinol transfer to the mammary gland via RBP, there is an additional way through which this nutrient is transferred. As this increase was observed in postprandial conditions, it is believed that this additional mechanism occurs via chylomicrons, as demonstrated in animals. Thus, it is suggested that vitamin A supplementation should be administered close to mealtimes to achieve active participation of chylomicrons and consequently, better utilization of the supplied vitamin.

According to Blaner et al., approximately 60% of vitamin A is transferred to the mammary gland by chylomicrons and relies on lipoprotein lipase (LPL) binding sites and on the hydrolysis of retinyl esters of chylomicrons through the action of this enzyme, whose activity increases during lactation. A higher percentage of ingested vitamin A is preferentially targeted to the mammary gland in lactating women, in contrast to what occurs in the absence of lactation states. It is also likely that a greater transfer of vitamin A to the mammary gland occurs due to an increase in RBP-retinol breast receptors to form colostr milk. This evidence corroborates the results obtained in the present study, which showed an increase in the amount of retinol after a meal and supplementation.

The vitamin A released during the hydrolysis of retinyl esters may be re-esterified for human breast milk secretion or stored in epithelial cells, which explains the maintenance of high levels of vitamin A in human milk hours after supplementation, as observed in this study. This occurs with the participation of the enzyme that esterifies retinol and is present in breast tissue, called acyl-coenzyme A: retinol acytransferase (ARAT). In addition, liver stores of vitamin A are also increased after supplementation and contribute to higher levels of retinol in breast milk.

Thus, it is suggested that vitamin A supplementation during postpartum increases the percentage of retinol in colostr milk, which, in postprandial conditions, occurs through the contribution of chylomicron retinyl esters produced as a consequence of the meal. Chylomicron remnants are cleared mainly by the liver, but extrahepatic uptake of

Table 2  Correlation between retinol concentrations in the milk of mothers under fasting and postprandial conditions, before and after vitamin A supplementation.

<table>
<thead>
<tr>
<th>Correlation (r)</th>
<th>BS, fasting</th>
<th>BS, postprandial</th>
<th>AS, fasting</th>
<th>AS, postprandial</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS, fasting</td>
<td>1.00</td>
<td>0.65</td>
<td>0.50</td>
<td>0.28</td>
</tr>
<tr>
<td>BS, postprandial</td>
<td>0.65</td>
<td>1.00</td>
<td>0.32</td>
<td>0.26</td>
</tr>
<tr>
<td>AS, fasting</td>
<td>0.50</td>
<td>0.32</td>
<td>1.00</td>
<td>0.65</td>
</tr>
<tr>
<td>AS, postprandial</td>
<td>0.28</td>
<td>0.26</td>
<td>0.65</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Spearman’s correlation.
BS, before supplementation; AS, after supplementation.

a. 0.10 < r < 0.29 (weak), 0.30 < r < 0.49 (moderate), 0.50 < r < 1.00 (strong), according to Cohen.
the remnants are important in the delivery of vitamin A to mammary tissue, being transported by RBP.

As shown in Table 2, 31 the statistical analysis demonstrated a strong correlation between the levels of retinol in colostrum milk of women before and after the meal on the first day, i.e., before maternal supplementation with vitamin A. However, the levels of retinol under postpartum condition on the following day after the administration of vitamin A supplement showed a weak correlation with fasting retinol levels on the first day.

One explanation for the above-mentioned weak correlation is that there appears to be a limit for the increase in the levels of micronutrients in breast milk, which may occur due to saturation of the proteins involved in transfer pathways of retinol from blood to the mammary gland (lipoproteins and RBP), as well as the enzymes involved in re-esterification and secretion of vitamin A in colostrum. Such situation would prevent an excessive increase of retinol in milk, indicating the existence of an adaptive mechanism to prevent the passage of excessive amounts of retinol to breast milk, thus protecting the baby against the toxicity of this micronutrient.

It is known that the concentration of retinol in human serum is greatly controlled by liver homeostasis mechanisms. On the other hand, this study showed that the biochemical state of retinol in breast milk can be modified according to dietary changes. Thus, for the diagnosis of the nutritional status of vitamin A, it is necessary that the milk collection is performed under fasting conditions.

This study observed an increase in retinol levels in human colostrum under postpartum conditions and after maternal supplementation with retinyl palmitate. This indicates that the mechanism of retinol transfer to the mammary gland via chylomicrons, already known in animals, also probably exists in humans. Furthermore, it was possible to verify the effectiveness of maternal supplementation, indicating that the supply of high doses of vitamin A to women in the immediate postpartum period, and especially after a meal, is an effective strategy to improve the nutritional status of retinol in lactating women and their infants.

**Conflicts of interest**

The authors declare no conflicts of interest.

**References**

23. Bezerra DS, de Araujo KF, Azevedo GM, Dimenstein R. A randomized trial evaluating the effect of 2 regimens of maternal


