Retinol and Fat from Breast Milk of Brazilian Mothers at High Risk for Food Unsafe

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Abstract

Promotion and protection of breastfeeding is an important strategy in prevention of Vitamin A (Retinol) deficiency in childhood. Breast milk retinol's allows us the chance to reach the maternal nutritional status and go over infant status. This study aimed to know the retinol and fat content in mature breast milk from Nova Cruziero's population, Brazil. It was analyzes 63 samples of breast milk that belonged to 81 children. The fat content was reached by crematocrit procedure and Retinol content by HPLC. Others datas had gotten through semi-structured pre-coded questionnaires. Statistics analyses were performed non-parametric for paired samples. The median of Retinol was 0,62 µg/100mL and 1,7µg/100mL from breast milk before and after the infant had breastfed. The samples were considered statistically different (p<0,001) for Retinol contents and for fat contents (p<0,001). The results shows that Vitamin A's content after the suck one , in each class of alimentary security, are strongly bigger than the content before suck (The Alimentary Security p=0,005; Light Unreliability p < 0,001; Moderate Unreliability p < 0,001; Deep Unreliability p < 0,001). The results suggest that the breast milk from the end of the suck provides greater retinol ingestion; breast milk before feed allows to reach The Brazilian Scale of Unreliability Alimentary. Although it is important to guide mothers to not interrupt the suck one and not to limit the manual expression only to initial breast milk.

ABBREVIATIONS

R: Retinol; VAD: Vitamin A Deficiency; BSUA: Brazilian Scale of Unreliability Alimentary

INTRODUCTION

Breast milk is the complete food for the infant. Provides energy and nutrients in appropriate amounts for good nutrition during the first months of life [1]. The promotion and protection of breastfeeding is an important strategy for prevention of various diseases, especially in children who live in communities of low socio-economic conditions [2]. The lactation stages produce milk of different composition, classifying breast milk: colostrum (thick, yellowish fluid produced three to six days after birth), transitional milk (seven to 15 days after birth) and mature milk (produced in continuity with transitional milk) [3]. Its composition is relatively constant, although the content of some nutrient varies significantly throughout the lactation during the day and even along a single feed [4]. This occurs, for example, with Vitamin A [5,6]. The content of Vitamin A in breast milk can also be influenced by the time of feeding, since the latter are richer in fat, involved in nutrient uptake and transport of vitamin A [7,8].

Vitamin A is a fat-soluble compound and its form of alcohol is called Retinol. Several retinol precursors' compounds are present in our diet, including the carotenoid stand out. Vitamin A also preformed is present in certain food sources, especially in the liver (stock in mammals) and milk (source for infants). The bioavailability of these different compounds is well known and equivalent forms are established that relates the measurement.

Vitamin A deficiency (VAD) is a major public health problem in developing countries. This deficiency is the leading cause of blindness could be prevented in children as well as being a factor that increases morbidity and mortality in the setting of infection [9]. Taking into account the nutritional demands increased during pregnancy and lactation, women in these phases, with marginal diets are more susceptible to VAD, thus integrating an epidemiological framework that can affect their children [10]. Assessment of vitamin A status of individuals and populations requires specific methodology and resources. Applications for laboratory research for measuring vitamin A and other fat-soluble vitamins have increased significantly [11].

The assessment of vitamin A status is done by measuring serum retinol concentration; however, this simple method is not a good indicator of hepatic retinol stocks, which usually needs...
to be assessed on interventions. Develop less invasive methods; more responsive to the determination of vitamin A status in lactating women helps to identify groups at risk of vitamin A deficiency. This could facilitate the evaluation of the effectiveness of supplementation programs and targeting specially these lactating women [12].

The concentrations of vitamin A in breast milk provide unique chance to determine the nutritional status of lactating and extrapolate this to the infant [13,14]. Values less than or equal to 1.05 mmol Retinol / L of milk or less than or equal to 8g (28 nmol) of Retinol / g milk fat are considered low in humans. Therefore, the dosage of Vitamin A in milk is a suitable alternative for population studies since breast milk collection is less invasive, and generally easier, more culturally acceptable than blood collection. Furthermore, samples do not need to be processed in the field [15,16]. Samples can be collected and kept under refrigeration for further analysis in an appropriate place [17].

The objective of this study was to determine Retinol's and fat content in breast milk from lactating mothers of the population of Novo Cruzeiro city, Mucuri Valley, Minas Gerais state, Brazil. In addition, to assess socio-economic, environmental and food insecurity status VAD-related in nursing mothers.

**MATERIALS AND METHODS**

**Location and sampling**

This cross-sectional population randomized study was conducted in Novo Cruzeiro, Mucuri Valley, northeast of the state of Minas Gerais. It occupies an area of 1701 km², with 30,199 inhabitants and has 2937 residents between zero and three years. The city is characterized by low human development index of 0.571 in 2010 by United Nations Development Programme. Household sampling was performed according to a study that evaluated food insecurity, nutritional status, food consumption and iodine deficiency in schoolchildren at the same city [19,20].

All lactating women who were producing mature milk from randomized households responded to the questionnaire to collect socio-economic data. Samples of breast milk were collected after 4h of woman’s fasting and babies’ fasting too. Breast milk composition changes from the beginning of a feeding to the end, diurnally, from day to day, and with the onset and progression of lactation. Fat and retinol content may also follow this variation. For this reason breast milk samples were also collected after at least 10 minutes of breastfeeding.

The main study was based on the number of Novo Cruzeiro’s children (n=5,696). A sample with 1,009 children from 6month-12years old were analyzed through the questionnaire. From these, 136 children were 6-24 months old. From the main study sample, a sub-sample of 63 nursing mothers produced 61 breast milk’s samples to analyses. These nursing mothers belong to 81 sample, a sub-sample of 63 nursing mothers produced 61 breast milk’s samples to analyses. These nursing mothers belong to 81
data analyses

**Measuring retinol**

In breast milk, vitamin A is present in retinol’s esters form. Therefore, a saponification step to free molecules of Retinol was needed [23]. A solution was prepared with Retinol Sigma® (C₂₀H₃₀O) batch 1384515, to construct the standard curve and used as internal quality standard. A solution of ® Retinyl palmitate (C₃₀H₄₀O₂), batch 047k440 was prepared for retinol’s esters elimination according to retention time of each substance.

Samples reached room temperature after 2 hours and they performed water bath at 36°C for 5 min, vortexed for 10 seconds. 500 mL breast milk aliquots were added to 100µL Retinyl palmitate standard at 0,03546µmol/L.

BHT alcohol solution at 20 mg / mL and 12.5 mol/L potassium-hydroxide was added. Samples were immersed in water bath at 80°C under dark for 25 min. Samples reached room temperature again and n-Hexane-Toluene 1:1 were added and homogenized. Samples were centrifuged at 4500g for 3 minutes. The organic phase was removed with a Pasteur pipette and dried under nitrogen.

Before the samples injection in HPLC, 210µL of methanol / water 89:11 (Merck methanol for HPLC) were added in each sample. The chromatograph used: Shimadzu LC-10AT HPLC® equipped with SPD-M10A detector diode array, and C18 reverse phase column. (140x4. 6 mm 0.5 mm particle size). 20µL of each sample was injected under flow rate of 2.5 ml / min. [21,22].

The amount of retinol in each sample was established by comparison with the retention times and areas of the respective standards. Standard concentrations were confirmed by the specific extinction coefficient in methanol / water (89:11) at wavelength of 325 nm [24]. This method allows to separate the esters of retinol. To add internal standard can avoid errors induced by solvent evaporation and oxidative conditions.

Data were analyzed with SPSS. Retinol content were used for statistical analysis followed the Student t test, the difference between means, considered significant when p <0.05. Retinol values are expressed as mean and standard deviation, and to test the differences between the means of numerical data, normality test was done and followed it to the non-parametric analysis of paired samples.

Recommended daily Retinol for infants was based on Dietary Reference Intake referring to 400 mg of Retinol [25].
RESULTS AND DISCUSSION

We identified 63 nursing mothers who were producing mature milk and it was analyzed 61 breast milk samples (they report to 81 randomized children). Only 2 mothers couldn’t collect enough breast milk for analysis (Figure 1). There were 5 mothers breastfeeding more than one children up to 60 month old.

Birth weight mean and standard deviation were 3244.4 kg and 420.4 kg, respectively. Prevalence of exclusive breastfeeding was 47% (38/81).

Results of 10 samples were registered twice: one for each kid up to 24 months old. 71 valid data pairs had Retinol measured (µg/100mL breast milk) and they are presented in Figure 2.

After feeding Retinol’s content is consistently larger than samples collected before feeding. Same behavior could be observed when fat was measured. Considering that Retinol is liposoluble, this behavior was expected. The relationship between retinyl acetate and lipid transport in body metabolism is widely known [26] (Figure 3).

In the 1990s, United States developed an instrument that assesses food insecurity [27], subsequently modified and adapted in several countries [28,29]. The relation between Retinol in mature milk before and after feeding and the state of food insecurity is shown in Figure 4. Retinol’s values before feeding and food insecurity hold a negative correlation. However, the measures after feeding did not suggest any trend in the level of food insecurity. It also shows that retinol’s content after feeding are significantly larger than before breastfeeding, in each class of food security (food safety, p = 0.005; light insecurity, p <0.001; moderate insecurity, p <0.001; serious insecurity, p <0.001, respectively) (Table 1).

Retinol concentration and fat levels are higher at the end of the breastfeeding. Retinol concentration decreases during occurrence of any disease and may not reflect the true status of vitamin A in a population with high prevalence of comorbidities [30]. This fluctuation in Retinol’s content in serum seems not reach the breast milk – with or without morbidity [31]. Retinol level does not change even if daily intake Vitamin A is provided. To analyze Retinol’s content in human breast milk is demanding to standardize best time of collection and best storage conditions. Especially for comparison purposes between different populations socio-economically [32]. Thus the comparison between the populations of developing countries and developed

Figure 1 Sampling. Selection of nursing mothers producing mature breast milk. Sampling of children up to 12 years old from the families randomized in this study.

Figure 2 Retinol in mature breast milk before and after children’s feeding. Novo Cruzeiro, Minas Gerais – Brazil.

Figure 3 Fat content according to Lucas Test in mature breast milk before and after children’s feeding. Novo Cruzeiro, Minas Gerais – Brazil.
Moderate or severe food insecurity: 86.4%; which has a positive relation with the values of Retinol concentrations before the infant feeding with mothers under 4h fasting? According to WHO less than 20% of the samples had sufficient Retinol for growth and development of infants. To reach Retinol's content is helpful in detecting poor communities / risk.

The fat content was lower before breastfeeding. However, this number does not distinguish the food security population from the food insecure population. This is unfortunate since the quantification of fat content is a mandatory practice in Brazil's human milk banks. If the fat content allowed to infer about the nutritional status of the mother and her family, it would be possible to find families most exposed to a lack of adequate food for children’s growth and development (Table 2).

After 15 minutes of breast-feeding babies refused the mother’s breast. Breast milk samples were collected in schools and health units, but also occurred in remote places such as corrals, chapels and small roads at rural area. Both mother and babies were not comfortable in these places. Older children's crying may have disturbed the baby and so babies sucked the breast with less intensity. They did not release the breast for at least 10 minutes but also did not maintain the same suction pattern throughout this time.

We must also consider that although more than 95% have electricity, only half have home refrigerator. What complicates the storage of foods, especially perishables ones such as vegetables, fruit and meat, rich in vitamins. Over 95% of mothers answered the questionnaire, which leads us to believe that they are responsible for home basic care children education. However, 67% of them consider the father as being the “family boss”. The unemployment rate in this region is more twice as high as in Brazil (23%) - which was expected. The Mucuri Valley is one of the “poverty pockets” in Brazil. 65.7% of these families live with less than the minimum wage, which explains to some extent the findings of food insecurity. They spend with food in average U$131,92 / month and their income in average were U$ 228,91 Dollars (Brazilian currency R$ 219,00 and R$ 380,00 Reais respectively). More than 70% live in rural areas. In this same population, it was determined significant prevalence of iodine deficiency [20].

85.2% have less than 8 years of education. 76.5% receive some kind of government aid. This may be a factor of influence. These families are expected to maintain these government benefits because they have no other source of income or employment.

CONCLUSION

It is possible to extrapolate the nutritional status of lactating women nutritional status to the entire population according to Retinol’s content in mature breast milk. Health services should incorporate into their routines the evaluation of Retinol content in human breast milk to find scores related to nutritional conditions of the poorest strata of the population. This would be a way of knowing who are food insecure families. With this non-invasive data, it would be possible to develop public policy strategies to ensure that children in developing countries receive adequate conditions for their growth and development. It would be a biochemical tool for measuring hunger and misery that would not allow middle-income families to receive government aid that should be directed only to those who really need it.

Vitamin Supplementation programs have contributed to the reduction of under-five mortality rates, but by itself does not solve

Table 1: Retinol Content (µg/100mL) and fat content (%) in mature breast milk before and after feeding. Novo Cruzeiro, Brazil.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>1st Quartile</th>
<th>Median</th>
<th>3rd Quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol Content before feeding (µg/100mL)</td>
<td>71</td>
<td>0,39</td>
<td>0,62</td>
<td>1,15</td>
</tr>
<tr>
<td>Retinol Content after feeding (µg/100mL)</td>
<td>71</td>
<td>1,47</td>
<td>1,70</td>
<td>2,31</td>
</tr>
<tr>
<td>Fat content before feeding (%)</td>
<td>71</td>
<td>0,82</td>
<td>2,09</td>
<td>7,59</td>
</tr>
<tr>
<td>Fat content after feeding (%)</td>
<td>71</td>
<td>3,33</td>
<td>5,71</td>
<td>7,59</td>
</tr>
</tbody>
</table>

Table 2: Families classification in Novo Cruzeiro, Brazil, according to the Brazilian Food Insecurity Scale (BFIS).

<table>
<thead>
<tr>
<th>Brazilian Food Insecurity Scale</th>
<th>n</th>
<th>Relative value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food security</td>
<td>11</td>
<td>13,6</td>
</tr>
<tr>
<td>Food insecurity</td>
<td>70</td>
<td>86,4</td>
</tr>
<tr>
<td>Light insecurity</td>
<td>39</td>
<td>48,1</td>
</tr>
<tr>
<td>Moderate insecurity</td>
<td>18</td>
<td>22,2</td>
</tr>
<tr>
<td>Serious insecurity</td>
<td>13</td>
<td>16,0</td>
</tr>
</tbody>
</table>

becomes inappropriate.

Table 4: Brazilian Food Insecurity Scale.

- Before Breastfeeding
- After breastfeeding
- Food Security (1); Light Food Insecurity (2); Moderate Food Insecurity (3); Severe Food Insecurity (4).
the underlying problem of inadequate dietary intake of vitamin A in preschool children in developing world. The proposed study helped identify risk populations with regard to food and nutrition. Identification and implementation of health actions directed to this population allows plans and / or rehabilitation projects and can still adapt the best treatment for the reality of the population and Brazilian health policy.

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