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## ORIGINAL ARTICLE

# *Retinol levels in human colostrum: influence of child, maternal and socioeconomic variables*

**Roberto Dimenstein,[1] Janicéia L. Simplício,[2] Karla D.S. Ribeiro,[2] Illana L.P. Melo[2]**

### Abstract

**Objectives:** To determine colostrum retinol levels in breastfeeding women from the city of Natal, state of Rio Grande do Norte, Brazil, and to investigate the relationship between retinol levels in colostrum and child, maternal and socioeconomic variables.

**Methods:** Forty-two healthy women aged 18 to 39 years were evaluated 48 hours after delivery. Socioeconomic, anthropometric, and dietary data were collected by means of a questionnaire. Body mass index was used to determine nutritional status. The Virtual Nutri software was used to analyze data on daily intake of vitamin A. Retinol levels were determined in 2 ml of colostrum using high-performance liquid chromatography.

**Results:** Most women were of low socioeconomic and educational backgrounds. Vitamin A intake was adequate in 55% of the women (mean intake = 1,398.8 µg RE/day). The mean amount of retinol in colostrum was 93.1 ± 51.1 µgRE/100 ml. No significant differences were found in terms of milk retinol levels for the variables income ($p = 0.503$), educational level ($p = 0.708$) and birth weight ($p = 0.499$). However, a statistically significant difference was observed for nutritional status during pregnancy (0.016).

**Conclusions:** The lack of influence of socioeconomic factors on colostrum retinol levels suggests the existence of an adaptive mechanism of the mammary gland to maintain adequate retinol levels that meet the infant's daily needs.

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1. PhD. Associate professor, Department of Biochemistry, Universidade Federal do Rio Grande do Norte (UFRN), Natal, RN, Brazil.
2. Nutrition student, Universidade Federal do Rio Grande do Norte (UFRN), Natal, RN, Brazil.

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## Introduction

Vitamin A is a micronutrient that is essential to health. Dimenstein[1] points out that this vitamin is involved in reproduction, the visual cycle and cellular differentiation, which, in turn, affects physiological processes such as growth, fetal development and the integrity if the immune system. Due to its role in embryo development and in the normal differentiation of epithelial tissues, vitamin A is of fundamental importance during periods of growth and development, such as during gestation and lactation.[2,3]

Vitamin A deficiency is a significant public health problem and is the main cause of permanent blindness accompanied by death among children in developing countries.[4] It also contributes to a significant increase in rates of infant mortality and morbidity associated with infectious processes.[5] The primary factor which leads to reduced vitamin A levels in children is the absence of maternal breastfeeding during the first six months of life,[6] whether exclusive or complemented.[7] In addition, children who are exclusively breastfed depend entirely on the concentration of vitamin A and the volume of their mothers' milk to meet their requirements.

Human colostrum, defined as the first lactic secretion of the nursing mother until the 7th day postpartum,[8] and is particularly rich in vitamin A. It can reach concentrations of approximately 200 µg/100 ml, being, therefore, an excellent dietary source of this vitamin during the first days of the child's life.[9]

In Brazil, little work has been published on the effects of maternal socioeconomic status and health indictors on the levels of vitamin A in colostrum. The objective of this study is to determine retinol levels in the colostrum of nursing mothers resident in the city of Natal (RN) and their relationship with certain variables related to the socioeconomic and health status of mother and child.

## Methods

The study was approved by the Committee for Ethics in Research of the Universidade Federal do Rio Grande do Norte. The sample was selected by convenience, with 42 nursing mothers taking part aged from 18 to 39. They were seen at the Escola Januário Cicco Maternity Unit, Natal-RN, during the period between January and March 2002, and were breastfeeding exclusively. The mothers were selected to meet the following criteria: resident in the city of Natal-RN, normal delivery, full term gestational period, single conception pregnancy and absence of chronic disease during pregnancy. After the mothers had given informed consent, questionnaires were filled out to obtain data on socioeconomic, anthropometric and dietary factors. The data on nutritional status was obtained from prenatal care records. Anthropometric nutritional status was calculated using the Rosso weight-gain chart and weight/gestational age curves.[10] Thus, with the aid of a ruler, the height of the expectant mother can be related to the ideal value for her weight. The value is then applied to the diagram and correlated with gestational age. Both instruments allow maternal weight gain to be established and the mothers classified as of normal weight normal, overweight and low weight. The socioeconomic variables studied were level of education and income. Data on income was expressed in terms of multiples of the national minimum monthly salary received by the family and divided to represent income per capita. The cut-off for a definition of a low income was set at 0.5 minimum salaries per capita, keeping in mind that when the minimum salary was created by Brazilian legislation it was intended to meet the basic needs of a "standard" family. All colostrum samples were obtained within 48 hours of birth, by manual expression of both breasts, twice during the day (morning and evening) to reduce variations that occur during the day, until a volume of 2 ml of milk was reached. The milk was collected in a polypropylene tube duly protected with aluminum foil to impede vitamin A degradation from the action of light. The samples were stored at -20 °C until analysis.

Dietary investigation to establish vitamin A nutritional history was by dietary recall of the last three months of pregnancy, and in order to calculate the resultant ingestion of vitamin A in the diet Virtual Nutri version 1.0 for Windows-1996 from USP[11] was used. Certain alterations were made to vitamin A concentrations for some foodstuffs according to the 1999 Food Composition Table, Brazilian Institute of National Statistics and Geography - IBGE.[12] The estimated total vitamin A was compared with dietary reference values (770 µgEqR/day for Vitamin A), from the Food and Nutrition Board.[13] No correlation was sought between vitamin A ingestion data and colostrum retinol concentrations, since a historical dietary enquiry is not the best instrument for analyzing micronutrients.

Retinol was extracted from the colostrum by the method described by Giuliano et al.,[14] the phases were separated and hexane evaporated in a nitrogen atmosphere, in a water bath at 37 °C. Extracts were re-suspended in 1 ml of methanol (HPLC grade) and centrifuged for 1 minute. The retinol concentration of the samples was measured by HPLC with a Shimadzu LC-10 AD Chromatograph, coupled to a Shimadzu SPD-10 A UV-VIS Detector and a Shimadzu C-R6A Chromatopac Integrator with a Shim-pack CLC-ODS analytical conditions column (4.6 mm x 25 cm). The chromatogram developed under the following mobile phase conditions: methanol 100% and flow 1.0 ml/min. The retinol in the samples was identified and quantified by comparison with their respective standard retention times and areas. Standard concentrations were confirmed by the specific extinction coefficient (ε1%, 1 cm = 1850) in absolute ethanol and with a wavelength of 325 nm.[15] For this study the following cut-off points were used for colostrum retinol levels: < 30 µg/100 ml (low retinol level); 30 59 µg/100 ml (low level in relation to mature milk) and ≤ 60 µg/100 ml (normal level for colostrum).

Data was processed by the CONSULEST laboratory at the Department of Statistics of the Universidade Federal do Rio Grande do Norte. Numerical data was processed for linear correlation, while the influence of socioeconomic and maternal health variables on colostrum vitamin A concentrations were subjected to non-parametric analysis of variance by the Kruskal-Wallis test. Retinol values were expressed as mean and standard deviation and to test the differences between the averages of numerical data the Student *t* test was used. Differences at $p < 0.05$ were considered significant.

## Results

According to the questionnaire on socioeconomic status and mother-baby health, the majority of nursing mothers had a per capita family income of less than 0.5 minimum salaries (55%), 74% had completed the first level of national education and 90% had given birth to babies weighing < 2,500 g. Employing Virtual Nutri[11] to analyze vitamin A ingestion, it was observed that 55% of the mothers presented adequate vitamin A consumption, with an average ingestion of 1,398,8 µgRE/day, according to the DRI.[13] When anthropometric nutritional status of the expectant mothers was analyzed by means of the weight/gestational age curve, it was found that 45% of the expectant mothers were within the limits of normality (Table 1).

The average of the values for retinol found in colostrum was 93.1 + 51.1 µgRE/100 ml, and when analyzed by cut-off points, 71% of the nursing mothers had ideal values or values above 60 µgRE/100 ml (Table 2). Women who were underweight, normal and overweight during pregnancy had average retinol levels of 63.3 + 37.9, 95.6 + 43.9 and 116.5 + 57.4 µgRE/100 ml, respectively (Table 3). Mothers with low incomes had an average of 99.5 + 54.6 gRE/100 ml, and those with high incomes 85.4 + 46.8 µgRE/100 ml. However no significant differences were found between retinol levels and variables relating to socioeconomic status and mother-baby health, except when the group that was underweight during pregnancy was compared with the overweight group ($p < 0.05$) (Table 3).

**Table 1 -** Distribution of the 42 nursing mothers being seen at the Maternidade Escola Januário Cicco according to socioeconomic status and mother-baby health

| | n | % |
|---|---|---|
| **Socioeconomic status** | | |
| **Family income per capita** | | |
| ≥ 0.5 minimum salary | 19 | 45 |
| < 0.5 minimum salary (low income) | 23 | 55 |
| **Level of education** | | |
| Unliterate | 5 | 12 |
| Incomplete elementary school | 16 | 38 |
| Elementary school | 10 | 24 |
| High school | 11 | 26 |
| **Health variable** | | |
| **Birth weight** | | |
| ≥ 2,500 g | 38 | 90 |
| < 2,500 g | 4 | 10 |
| **Gestational nutritional status*** | | |
| Low weight | 11 | 26 |
| Normal weight | 19 | 45 |
| Overweight | 12 | 29 |
| **Dietary intake of vitamin A†** | | |
| Apropriate (≥ 770 µg/day) | 23 | 55 |
| Inappropriate (< 770 µg/day) | 19 | 45 |

* Anthropometric assessment, during the last three months of pregnancy, by means of Rosso weight/gestational age curves.[10]

† Dietary Reference Intake (DRI): 770 µg/day.[13]

**Table 2 -** The average of the values for retinol found in colostrum of the nursing mothers being seen at the Maternidade Escola Januário Cicco, Natal (RN)

| Retinol | Total group (n = 42) |
|---|---|
| Average+SD ( µg/100 ml) | 93.10+51.11 |
| > 60 µg/100 ml (%) | 71.43% |
| 30 - 59 µg/100 ml (%) | 21.43% |
| < 30 µg/100 ml (%) | 7.14% |

## Discussion

During pregnancy, ingestion and hepatic reserves of maternal vitamin A are essential to guarantee the transference of this micronutrient to the fetus, and are its first source of the nutrient.[16] The observed average vitamin A ingestion of women in developing countries (660 µgRE/day) is less than half that found in developed countries (1540 µgRE/day).[17] Among pregnant women registered with the public health system in Rio de Janeiro, average vitamin A ingestion was 2692 µgRE/day,[18] while in deprived populations in Campinas-SP, an average was found that was below that of the developing countries.[19] This study found an adequate average vitamin A consumption, in common with Ortega,[20] despite the majority of the nursing mothers belonging to the group with lower socioeconomic status. However, only 55% of the women were consuming sufficient quantities of the micronutrient to meet their needs (770 µgRE/day, DRI 2001), a fact which was also encountered by Moura[21] in São Paulo, indicating a low consumption of vitamin A rich foods on a populational level.

Maternal socioeconomic status has been implicated as being associated with vitamin A concentrations in milk. Newman[17] found that the level of this nutrient is lower in the milk of nursing mothers from developing countries when compared with that of women from developed countries. Studies of Swedish and Ethiopian women, privileged and under privileged, found retinol values of 40-53.2 and 28.3-33.2 µgRE/100 ml, respectively.[22] Income also affected retinol concentration in the milk of nursing mothers in Bangladesh.[23]

In Brazil, and certain countries in Latin America, vitamin A deficiency is considered a public health problem with a high frequency of sub-clinical manifestations.[24] This situation exists in populations that habitually ingest quantities close to minimum requirements or below the recommended levels and becomes of concern in critical situations, particularly during periods of growth and development, such as during pregnancy and lactation. Sub-clinical vitamin A deficiencies, common in our region, mask the real clinical condition without provoking hypovitaminosis A symptoms. This is perhaps the explanation for the absence of a correlation between low socioeconomic status and vitamin A concentration in

**Table 3 -** Relation between the mothers' nutritional status and the levels of retinol in the colostrum according to anthropometric assessment, birthweight, family income and level of education

| | Retinol µg/100 ml |
|---|---|
| **Anthropometric assessment*** | |
| Low weight (n = 11) | 63.35+37.98 |
| Normal weight (n = 19) | 95.55+43.96 |
| Overweight (n = 12) | 116.49+57.41 |
| **Birthweight†** | |
| Low weight (< 2,500 g) | 76.40+45.99 |
| Normal weight (≥ 2,500 g) | 94.86+51.87 |
| **Income ‡** | |
| Low | 99.53+54.60 |
| High | 85.37+46.78 |
| **Level of education§** | |
| Unliterate | 110.78+52.18 |
| Incomplete elementary school | 87.13+52.49 |
| Elementary school | 89.52+42.54 |
| High school | 97.00+60.14 |

* Significantly different averages (p = 0.016), Kruskall-Wallis test;
† Non significantly different averages (p = 0.498), Student's t test;
‡ Non significantly different averages (p = 0.503), Kruskall-Wallis test;
§ Non significantly different averages (p = 0.708), Kruskall-Wallis test.

breastmilk found in our study. Income and education, far from having the expected effect on retinol levels in colostrum, actually presented an inverse relationship with lower average concentrations among women with higher levels of education or income. This effect was also observed by Vitolo et al.[19] among women in Campinas - SP. Notwithstanding, the studies that found maternal socioeconomic status to have an influence on the concentration of vitamin A in milk were mainly carried out in populations living in extreme poverty, with a high frequency of clinical manifestations of hypovitaminosis A.

In addition the average colostrum retinol level was 93.1 + 51.1 µg/100 ml, which agrees with levels found by Macias[25] in Cuban women. Adopting the cut-off points already mentioned, we observed that only 7.1% of the nursing mothers presented a retinol deficiency (< 30 µg/100 ml), while the majority had levels above 60 µg/100 ml (Table 2). This data provides evidence that even in the face of poor socioeconomic conditions, colostrum vitamin A levels are maintained at an adequate level when compared with populations in better socioeconomic conditions. When maternal nutritional status is analyzed by anthropometric indicators, however, there are significant differences between the underweight and overweight groups, suggesting that the adaptive mechanisms that ensure breastmilk retinol levels, irrespective of socioeconomic conditions, are fragile and are probably associated with factors such as malnutrition. Accioly[18] did not observe significant associations between anthropometric values or nutritional status and the vitamin A levels of expectant mothers in Rio de Janeiro, drawing attention to qualitative dietary factors, which are very different in different regions of Brazil. This demonstrates that in a state of partial poverty, in contrast with extreme deprivation, vitamin A levels in breastmilk tend towards normality.

No alterations to colostrum retinol levels were observed among the nursing mothers studied in terms of socioeconomic variables and birth weight. This result suggests the existence of an adaptive mechanism maintaining mammary glandular levels of retinol at an adequate level to meet the daily needs of the infant. The variations that were observed in retinol levels between underweight and overweight groups should be better investigated in order to evaluate the influence of factors such as malnutrition and qualitative dietary factors.

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Corresponding author:
Roberto Dimenstein
Avenida Praia de Genipabu, 2100/1402
CEP 59094010 – Natal, RN, Brazil
Tel.: + 55 (84) 219.4340
E-mail: robertod@ufrnet.br