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Maternal supplementation with retinyl palmitate during immediate postpartum period: potential consumption by infants

ABSTRACT

OBJECTIVE: To assess the effect of maternal supplementation with a single dose of retinyl palmitate during the postpartum period, in order to provide vitamin A for the infant.

METHODS: A clinical trial was conducted in Natal (Northeastern Brazil), between March and December 2007, on 85 women distributed randomly into two groups. The postpartum supplements of retinyl palmitate consisted of a single dose of 200,000 IU (experimental group) and zero IU (control group). The retinol levels in milk were quantified using high performance liquid chromatography. Based on the retinol concentrations obtained in breast milk and through simulations, vitamin A consumption among infants 24 hours and 30 days postpartum was calculated.

RESULTS: The daily provision of retinol to newborns through colostrum, 24 hours postpartum, was 1.63 μmol for the controls and 2.9 μmol for the experimental group, taking adequate intake to be 1.40 $\mu\text{mol}/\text{day}$ and the milk volume consumed to be 500 ml/day. Thirty days postpartum, these values were 0.64 $\mu\text{mol}/\text{day}$ (controls) and 0.89 $\mu\text{mol}/\text{day}$ (experimental group), corresponding to a 39% increase in retinol concentration in the experimental group, in relation to the control group, or 64% of the recommendation for infants aged zero to six months.

CONCLUSIONS: Maternal supplementation with 200,000 IU of retinyl palmitate during the immediate post-partum period, and promotion of breastfeeding practices, are efficient for increasing the nutritional status of vitamin A for the mother-child pair.

DESCRIPTORS: Vitamin A Deficiency, prevention & control. Dietary Supplements. Maternal Nutrition. Infant Nutrition. Maternal and Child Health. Clinical Trial.

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Received: 07/15/2008
Revised: 12/06/2008
Approved: 01/23/2009

INTRODUCTION

Vitamin A is a micronutrient that is fundamental for growth, differentiation and completeness of the epithelial tissue, and it is essential during periods of major cell proliferation such as pregnancy and early infancy.^{4,19}

Around 127 million children of preschool age and seven million pregnant women around the world present vitamin A deficiency, and this is considered to be an important factor causing morbidity and mortality among child populations.²² Hypovitaminosis A is the main cause of permanent blindness

followed by death among children in developing countries.¹⁹ Infants' hepatic reserves of vitamin A are limited and therefore low at birth, because of the tendency for pregnant women's serum retinol levels to decrease, especially during the final trimester of pregnancy. Moreover, a selective placental barrier limits the transfer of this vitamin to the fetus in order to avoid possible teratogenic effects.^{4,20}

Hence, children's diets at birth and during the first years of life have repercussions throughout their lives. Since milk is the food most consumed during the initial stages of life, it is considered to be the most important source of vitamin A for multiplying newborns' hepatic reserves.¹⁹ Over the first six months of breastfeeding, 60 times more vitamin A is transferred from mothers to their children, compared with what is accumulated by the fetus over the nine months of pregnancy.^{12,a}

The recommendation from the Ministry of Health^b and the World Health Organization^c (WHO) is that children should be exclusively breastfed with maternal milk up to the age of six months, followed by complementary food associated with breastfeeding until at least the age of two years.

Thus, under ideal breastfeeding conditions, maternal milk is considered to be a major protective factor against vitamin A deficiency up to the age of two years, which is the period of greatest vulnerability to the development of this deficiency.¹⁰ Promotion and protection of breastfeeding is consequently an important strategy for preventing hypovitaminosis A during childhood. However, the contribution of maternal breastfeeding towards the offer of vitamin A depends on certain conditions such as the concentration of this vitamin in the milk, the quantity of milk consumed and infants' requirements for vitamin A.¹⁸ According to the Dietary Reference Intake, infants need 1.40 µg of retinol per day during the first months of life. This is the quantity required for vitamin A reserves to be accumulated and the clinical symptoms of deficiency to be impeded.⁸

However, if the maternal vitamin A status is poor, even infants that are breastfed may become deficient in vitamin A at the age of around six months.¹³

Studies on interventions to avoid such deficiencies, through supplementation with retinyl palmitate, have been conducted worldwide with successful results. Maternal supplementation with megadoses during the immediate postpartum period is an intervention that has

been greatly used in areas that are at risk of vitamin A deficiency,²¹ including Brazil. Since 2002, through the Brazilian Ministry of Health's ordinance no. 2160, dated December 29, 1994, vitamin A supplementation through megadoses (200,000 IU) has been administered orally to puerperae living in the states of the northeastern region of Brazil, along with those living in the Jequitinhonha valley (Minas Gerais) and in three municipalities of the state of São Paulo (Nova Odessa, Hortolândia and Sumaré).^a This measure is a potentially effective strategy for simultaneously improving the vitamin A status of women and their infants.²¹ Through recommending supplementation as an immediate preventive measure for combating vitamin A deficiency among puerperae and infants, it is hoped that its action will be long-lasting, i.e. for at least the first six months of life.^b However, studies evaluating the effects from such interventions are still rare in Brazil.

In this light, the aim of the present study was to evaluate the effect of maternal supplementation with a single dose of retinyl palmitate during the postpartum period, in order to supply vitamin A to the infant at the concentration of retinol in maternal milk.

METHODS

This was a clinical trial conducted among parturients at a public maternity hospital who lived in the urban area of the city of Natal, Northeastern Brazil, which has approximately 244,743 inhabitants, corresponding to 34% of the population of the municipality. The subjects' mean monthly income was 2.92 minimum salaries.^d

The sample size was calculated inferentially, using means and dispersion measurements from a previous study.³ The standard deviation of the maternal retinol during a postpartum month was taken to be not greater than 0.52 µmol/l.³ Consequently, it would be necessary to recruit 42 women in each group, in order to detect a difference of 0.35 µmol/l, with a power of 80%, confidence interval of 95% and loss from follow-up of 25%.

Sampling was conducted according to convenience. From this, 113 healthy parturients aged 18-40 years were recruited, as volunteers. They gave birth to a single infant at full term, without malformations, between March and December 2007. None of them had received any vitamin A supplementation during their pregnancies. At an interview, they answered a questionnaire that sought information about their prenatal period,

^a Dolinsky M, Ramalho A. Deficiência de vitamina A: uma revisão atualizada. *Compacta: temas em nutrição e alimentação* [internet]. 2003;4(2):3-18 [cited 2009 Apr 25]. Available from: <http://www.pnut.epm.br/compacta.htm>

^b Ministério da Saúde. Projeto Suplementação de Megadose de Vitamina "A" no pós-parto imediato nas maternidades/hospitais [Internet]. Brasília (DF); 2002 [cited 2009 May 25]. Available from: http://nutricao.saude.gov.br/mn/vita/docs/projeto_vita.pdf

^c World Health Organization, Collaborative Center for Diet and Nutrition in the Northeast I. Vitamin "A" during pregnancy and lactation. Recommendations and report from a consultation. Recife; 2001. (Micronutrient Series)

^d Prefeitura do Natal: Secretaria Municipal do Meio Ambiente e Urbanismo – SEMURB [cited 2009 Jun 29]. Available from: <http://www.natal.rn.gov.br/semurb/paginas/ctd-328.html>

delivery and clinical history. Some information was obtained by consulting their prenatal follow-up cards and their hospital medical files. Their anthropometric nutritional status during the pregnancy was assessed by means of the body mass index (BMI) during pregnancy, correlating the weight/height relationship with the gestational age (GA), based on information from the parturients' last prenatal consultation. To classify the adequacy of the BMI/GA ratio, the graph proposed by Atallah et al² (1997) was used.

The women were distributed randomly into two groups for this experiment. One group, named S1, received supplementation during the immediate postpartum period consisting of a single dose of 200,000 IU (60 mg) of retinyl palmitate. The other was a control group (named C) and did not receive any supplementation. Out of the 113 women recruited, 85 (75%) remained in the study until the end of the experiment. For group C, the aim was to keep up the number of participants so that this was at least 50% of the number of women in the group receiving supplementation, given that the control group was formed with the main aim of characterizing the study population.

While still in the maternity hospital, the puerperae in group S1 were supplied with a capsule of retinyl palmitate (200,000 IU + 40 mg of vitamin E).^a Twenty-four hours after this supplementation, maternal milk from all of the mothers was collected the next morning, after overnight fasting, by means of manual expression from a single breast that had not been previously sucked (24-hour milk). The first ejection of milk was discarded in order to avoid fluctuations in the retinol and fat content. These samples consisted of one to two ml of colostrum milk, and the aliquots were collected in polypropylene tubes protected from the light, with proper identification.

Thirty days after the delivery, a home visit was made and all the participants supplied a second milk sample on this occasion. The women in the control group received supplementation after providing a milk sample during the visit. The milk samples were transported under refrigeration to the Nutrition Biochemistry Research Laboratory, Department of Biochemistry, Biosciences Center of the *Universidade Federal do Rio Grande do Norte* (UFRN). The aliquot volumes were quantified and the samples were then stored at -20°C until the time when the analyses were performed.

All of the samples collected at the two times were analyzed in relation to the circumstances of the two groups: mothers without supplementation (C) or with supplementation with 200,000 IU of retinyl palmitate (S1). The intake model included the vitamin A

concentration in the maternal milk, the recommended quantity of maternal milk consumption and the vitamin A requirements for infants between zero and six months of age.⁸ For this age range and a milk consumption volume of 500 ml/day, the mean value for adequate intake is 1.40 $\mu\text{mol/day}$.⁸ These estimates from the literature¹⁸ were used to simulate and compare different circumstances, through providing a measurement of the potential benefits obtained from improvements in the maternal vitamin A status.

The data were expressed as μmol of retinol per liter of maternal milk. Retinol levels in the maternal milk greater than 1.05 $\mu\text{mol/l}$ were considered to indicate that the mother presented at least the minimum reserves required, since levels greater than this value are common in healthy populations without evidence of insufficient vitamin A in the diet.^{13,24} Thus, values $\leq 1.05 \mu\text{mol/l}$ were considered to indicate low retinol concentrations in the mature milk, thereby suggesting that the maternal intake of vitamin A was inadequate and that the infant was at greater risk of developing vitamin A deficiency. This situation is considered to be a public health problem when 10% or more of the infants have retinol levels below this value.²⁵

The extraction of retinol from the milk samples was performed in accordance with Giuliano et al⁷ (1992). The retinol concentration in the samples was determined using the high performance liquid chromatography (HPLC) method. A reverse phase system was used, followed by UV detection at 325 nm. The chromatograph used was the Shimadzu LC-10 AD, coupled to the Shimadzu SPD-10 A UV-VIS detector and the Shimadzu C-R6A Chromatopac integrator with the Shim-pack CLC-ODS (M) 4.6 mm x 25 cm LC column.

The chromatograms were run in the form of isocratic elution, with a 100% methanol mobile phase. The vitamin A retention time was 4.3 minutes at a flow rate of 1 ml/min. Retinol was identified and quantified in the samples by comparison with the retention time and area of the respective standard, at a wavelength of 325 nm.

The concentration of the standard was confirmed by means of the specific extinction coefficient (ϵ 1%, 1 cm = 1780) in pure ethanol, at a wavelength of 325 nm.¹⁴ The accuracy of the method was evaluated by means of an extraction recovery test, from which 95% recovery of the retinol acetate (internal standard) that had been added to the samples was obtained.

The precision was evaluated by means of the reproducibility test, in which triplicates of the same milk sample

^a Ministério da Saúde. Projeto Suplementação de megadose de vitamina "A" no pós-parto imediato nas maternidades/hospitais [Internet] Brasília (DF); 2002 [cited 2009 May 25]. Available from: http://nutricao.saude.gov.br/mn/vita/docs/projeto_vita.pdf

were measured for retinol for three alternate days. The values found presented variation of less than one standard deviation. The standard curve was produced with the reference standard of all-trans retinol (Sigma) at different concentrations ranging from 2 to 32 ng/20 µl. The detection and quantification limits were based on the linearity of the standard curve, and values of 0.1 µg/ml and 2 µg/ml, respectively, were obtained.

To process the data, the SPSS 13.0 software was used. The samples were subjected to the Kolmogorov-Smirnov test to assess whether the distribution met the normal curve. Comparisons between means were made using independent or paired t tests. The categorical variables at the baseline were analyzed using the χ^2 test. The data were presented as arithmetic means and standard deviations (sd) and, in all cases, two-tailed

analyses were used and the results were considered statistically significant when $p < 0.05$.

The study was approved by the Research Ethics Committee of the *Universidade Federal do Rio Grande do Norte* (Protocol no. 128/06). All the participants signed a free and informed consent statement.

RESULTS

The random enrollment gave rise to homogenous maternal characteristics (Table). The subjects' overall mean age was 24.5 years (sd= 5.3). It was observed that normal deliveries predominated (65%) and that most of the puerperae had already breastfed other children. Regarding the anthropometric nutritional status during pregnancy, most of them were eutrophic (44%).

Table. Characteristics of the parturients and newborns studied. Municipality of Natal, Northeastern Brazil, 2007.

| Variable | Control (n= 30) | S1 (n= 55) | Total (n= 85) |
|---|-----------------------------|----------------|------------------|
| Mother | | | |
| Age (years) | 24.5 (sd= 5.5) ^a | 24.9 (sd= 5.2) | 24.8 (sd= 5.2) |
| Parity (number of children) | 2.1 (sd= 1.2) | 2.1 (sd= 1.2) | 2.1 (sd= 1.2) |
| Gestational age (weeks) | 39.4 (sd= 1.1) | 39.0 (sd= 1.2) | 39.1 (sd= 1.2) |
| Type of delivery | | | |
| Normal [n (%)] | 18 (69) | 31 (56) | 49 (60) |
| Cesarean [n (%)] | 8 (31) | 24 (44) | 32 (40) |
| Gestational nutritional status^b | | | |
| Underweight [n (%)] | 3 (12) | 6 (15) | 9 (14) |
| Eutrophy [n (%)] | 10 (40) | 15 (39) | 25 (39) |
| Overweight [n (%)] | 8 (32) | 12 (31) | 20 (31) |
| Obesity [n (%)] | 4 (16) | 6 (15) | 10 (16) |
| Newborn | | | |
| Sex | | | |
| Female [n (%)] | 13 (46) | 25 (46) | 38 (46) |
| Male [n (%)] | 15 (54) | 29 (54) | 44 (54) |
| Weight (kg) | 3.2 (sd= 0.5) | 3.1 (sd= 0.5) | 3.2 (sd= 0.5) |
| Length (cm) | 47.2 (sd= 3.6) | 49.5 (sd= 2.3) | 48.6 (sd= 2.9) |
| Nutritional status (W/L)^c | | | |
| Malnutrition [n (%)] | 0 (0) | 2 (4,5) | 2 (3) |
| Risk of malnutrition [n (%)] | 0 (0) | 4 (9) | 4 (6) |
| Eutrophy [n (%)] | 18 (75) | 28 (64) | 46 (68) |
| Risk of overweight [n (%)] | 4 (17) | 8 (18) | 12 (18) |
| Overweight [n (%)] | 1 (4) | 2 (4,5) | 3 (4) |
| Obesity [n (%)] | 1 (4) | 0 (0) | 1 (1) |

S1: Group supplemented with one megadose (200,000 IU) of retinyl palmitate

W/L: Weight for length

^a Mean (standard deviation)

^b Anthropometric nutritional status relating to the data from the last prenatal consultation (Atallah et al,² 1997)

^c Anthropometric nutritional status of the newborn. Frequencies were compared using the χ^2 test and mean values using the t test; no significant differences were found for $p < 0.05$.

Among the newborns, males predominated (54%) and the most frequent anthropometric nutritional status was eutrophic (64%), according to the weight/length ratio (W/L).

In evaluating the immediate effect of maternal supplementation with vitamin A, on the maternal milk, it was seen that the samples presented normal distribution. Furthermore, there was a statistically significant increase in mean retinol levels in the colostrum of the supplemented group: 3.22 (sd= 1.81) $\mu\text{mol/l}$ and 5.76 (sd= 2.80) $\mu\text{mol/l}$ ($p < 0.0001$), between time zero and 24 hours, respectively. This increase did not occur in the control group ($p = 0.69$), since this group presented baseline mean retinol levels of 3.31 (sd=1.40) $\mu\text{mol/l}$ at time zero and 3.26 (sd= 1.21) $\mu\text{mol/l}$ after 24 hours.

On the 30th day, analysis on the retinol values per volume of mature milk also indicated a significant difference between the means for the groups ($p < 0.05$): 1.28 (sd= 0.61) $\mu\text{mol/l}$ for the control group and 1.78 (sd= 1.00) $\mu\text{mol/l}$ for S1. Thus, the puerperae studied presented adequate retinol concentrations in the maternal milk at the two times investigated (24 hours and 30 days) (Figure 1).

Based on the retinol concentrations found in the maternal milk, in the different study groups and at their respective investigation times, it was observed that at the time of 24 hours, the daily supply of retinol to the newborn via the colostrum was 1.63 μmol and 2.9 μmol for the C and S1 groups, respectively. Thus, the retinol intake was considered adequate. Thirty days after delivery, these values were 0.64 $\mu\text{mol/day}$ (C) and 0.89 $\mu\text{mol/day}$ (S1), considering the same volume of milk consumed (Figure 2).

DISCUSSION

The women in the present study were adults and predominantly multiparous, with some characteristics similar to populations studied in Spain¹⁵ and Brazil (city of Rio de Janeiro).¹¹ The profile found was also similar to that observed by Dimenstein et al⁵ (2003) in Natal, Brazil.

The retinol concentration in milk forms a satisfactory alternative for evaluating the vitamin A nutritional status. This measurement not only is less invasive than blood collection,¹ but also gives information on the supply of this vitamin to infants²³ and predicts the vitamin A status, both for mothers and for their children.

Although the low socioeconomic condition of breastfeeding women and the condition of hypovitaminosis A represent a public health problem in Brazil,¹⁶ the retinol in maternal milk in the present study showed normal levels at all stages of lactation. The retinol concentration in the colostrum was greater than that

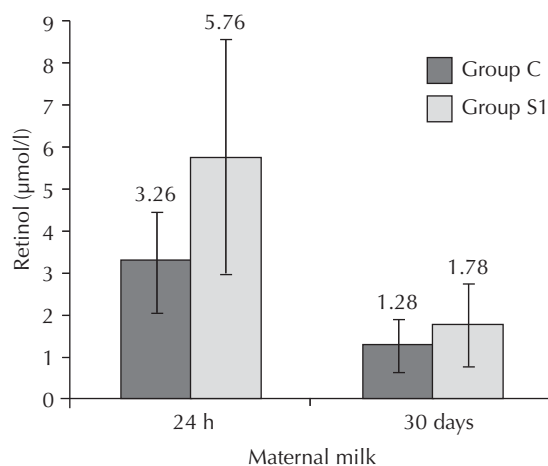


Figure 1. Retinol concentration in the breastfeeding women's milk at the two investigation times. Municipality of Natal, Northeastern Brazil, 2007.

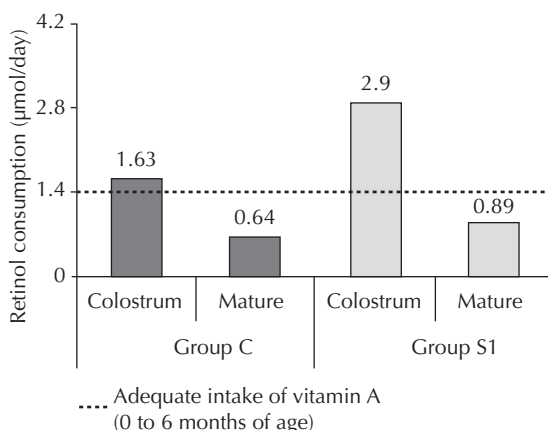


Figure 2. Daily retinol consumption by infants in the supplementation and control groups. Municipality of Natal, Northeastern Brazil, 2007.

of the mature milk, as reported in the literature.¹⁸ The mean baseline value for retinol in the colostrum was similar to what has been found among Cuban women⁹ and in developed countries.¹⁸ Furthermore, the retinol concentration in the milk was shown to be sufficient to meet the vitamin A requirements of the newborns and impede the development of hypovitaminosis A.

Maternal supplementation with 200,000 IU of retinyl palmitate increased the retinol concentrations in the maternal milk of group S1, thus agreeing with experiments conducted in Bangladesh¹⁷ and Indonesia.²¹ Twenty-four hours after the supplementation, the colostrum reached values capable of supplying more than twice the recommended supply of retinol to newborns (2.88 $\mu\text{mol/day}$), considering that during this period, the infants were consuming a mean volume of 500 ml of milk per day.⁸ It is likely that this situation

is advantageous, given that the colostrum has a fundamental role in the initial formation of the infants' hepatic reserves of vitamin A, prior to the decline in serum retinol levels to less than half, as usually occurs after one month of lactation.¹⁸

We observed that the supply of vitamin A in quantities sufficient to reach the recommended adequate intake for children did not extend to the mature milk. Thirty days after the supplementation, the retinol concentration in group S1 had increased the contribution of maternal milk towards supplying retinol to the children by 39%, compared with the control group. It came to represent 64% of the recommendation of the Institute of Medicine⁸ (2001) for infants from zero to six months of age with consumption of 500 ml/day.

However, Ross & Harvey¹⁸ (2003) suggested that the above mentioned volume of maternal milk corresponded to the daily consumption during the first week of life, and that this would increase to 620 ml over the first three months and to 660 ml from the third to the sixth month of age. In this way, the contribution of the maternal milk towards supplying retinol to the infants would increase to 57% and 79% in the control and S1 groups, respectively.

Adequate intake represents the mean daily intake of a nutrient and probably exceeds the requirements of most healthy individuals at a given stage of life, according to sex.⁸ The main factor leading to diminished vitamin

A levels in children is the absence of breastfeeding over the first six months of life,⁶ even though exclusive breastfeeding is recommended by WHO and the Brazilian Ministry of Health as the ideal strategy for ensuring growth and development, thereby diminishing the burden of morbidity during childhood.^{26,a} It also has important implications for maternal health.^b Thus, maternal supplementation with vitamin A probably benefits infant nutrition and avoids the potential toxicity of supplementation at high doses to the infants. The strategies for improving the vitamin A status of infants and preschool children include improvement of the vitamin A status of their mothers while they are lactating. Through this, the use of colostrum and exclusive breastfeeding for the first six months of life will be promoted, along with the addition of foods that are sources of vitamin A after the age of six months, while continuing to breastfeed until the child reaches two years of age.¹³

In this light, maternal supplementation with 200,000 IU of retinyl palmitate during the immediate postpartum period and the promotion of optimized breastfeeding practices are highly efficient for increasing infants' vitamin A consumption and for improving the vitamin A nutritional status of the mother-child pair. Both of these strategies need to be strengthened by increasing the levels of care and resources in order to achieve the greatest possible reductions in infant morbidity-mortality, with improvements in vitamin A status.

^a Ministério da Saúde. Secretária de Política de Saúde, Pan-American Health Organization. Dietary guide for children under two years of age. Brasília (DF); 2002. (Série A. Normas e Manuais Técnicos, 107).

^b Pan-American Health Organization. Breastfeeding [internet]. Jun. 2003 [cited 2008 Jun 22]. Available from: <http://www.opas.org.br/sistema/fotos/amamentar.pdf>

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