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Vitamin A intake of Brazilian mothers and retinol concentrations in maternal blood, human milk, and the umbilical cord

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Abstract

Objectives: To analyse intake of vitamin A (VA) and retinol concentrations in maternal blood, breast milk (BM), and the umbilical cord (UC) of newborns, and to determine the associations among these variables.

Methods: We performed a cross-sectional, epidemiological study of 180 mother–newborn dyads. Maternal and UC blood samples and BM were collected. VA intake by the mother over 30 days was assessed using a questionnaire.

Results: Mean retinol concentrations in maternal serum, the UC, and BM were 0.65 ± 0.27, 0.36 ± 0.18, and 2.95 ± 2.70 μmol/L, respectively. Retinol concentrations <0.70 μmol/L were found in 57.2% of maternal blood samples and in 94.9% of UC samples. A total of 27.9% of BM samples showed retinol concentrations <1.05 μmol/L. Mean VA intake by the mothers was

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1041.33 ± 1187.86 μg retinol activity equivalents/day and was inadequate (<550 μg retinol activity equivalents/day) in 44.7%.

Conclusions: High proportions of insufficient retinol concentrations were observed in the UC, maternal blood, and BM. A high percentage of pregnant women had inadequate VA intake. Mothers with insufficient serum retinol concentrations had newborns with lower retinol concentrations in the UC. Higher retinol concentrations were observed in maternal blood and the UC with a higher VA intake.

Keywords

Retinol, vitamin A deficiency, colostrum, umbilical cord, newborn, maternal blood

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Introduction

Vitamin A (VA) is present in several foods, such as preformed VA and pro-vitamin A carotenoids, which are dietary precursors of retinol. Preformed VA is found in foods of animal origin and pro-VA carotenoids are mainly found in dark green and yellow-orange fruits and vegetables, oleaginous fruits, and palm oil.[1] In pregnancy, retinoids are essential for metabolism and tissue growth of the mother. Retinoids are also necessary for growth, tissue maintenance, and embryo/foetal reserve formation.[2]

Vitamin A deficiency (VAD) is one of the major nutritional problems of public health in the developing world. VA mainly affects pregnant women and children, who represent periods of life in which nutritional demand is high and foods are deficient in VA.[3,4] The human embryo exclusively depends on retinol of the maternal circulation for its supply of VA. VA reaches the embryo by crossing the maternal–foetal barrier (i.e., the placenta and the vitelline sac). Generally, circulating retinoid concentrations reflect the maternal VA status, which is determined by stores and recent intake of VA. Therefore, changes in these concentrations affect the quantity of VA available for crossing the placenta and reaching the foetus.[5,6]

The foetus starts to accumulate VA only during the third trimester of gestation and transfer of retinol from mother to child is limited. Therefore, neonates are born with low VA reserves and approximately half the serum retinol concentrations compared with their mothers.[7,8] Consequently, appropriate retinol transfer through human milk (HM) is required during lactation to increase the VA reserves of the infant before weaning.[9,10]

During pregnancy and the breastfeeding period, the requirements of VA intake by the mother are increased. Because the neonate is fully dependent on its mother in terms of retinol supply before and after delivery (through the placenta and mother's milk, respectively), an adequate intake of this micronutrient by the mother is important.[11,12]

Therefore, the objectives of the present study were: 1) to examine VA intake of mothers and retinol concentrations in maternal blood, breast milk (BM), and the umbilical cord (UC) of newborns; 2) to describe VA intake in the last month of pregnancy and retinol concentrations in

maternal and newborn blood, and in BM; 3) to analyse the associations among these variables.

Methods

Study design, ethics, and study group

A descriptive, cross-sectional, epidemiological study was conducted at the largest public maternity hospital in Ribeirão Preto (São Paulo, Brazil), which provides care for an estimated population of 1,300,000 inhabitants. The sample size was calculated according to the recommendations of Scheaffer et al.[13] This calculation took into consideration a mean number of 290 deliveries per month at the maternity hospital and a 5-month period of data collection. A precision value of 5% and a 15% prevalence of VAD were estimated for Brazilian women of childbearing age,[14] which resulted in a sample size of 179.

Inclusion criteria were as follows: healthy women aged 18 years or older; and mothers of singleton foetuses born by vaginal or caesarean delivery after term gestation (gestational age of 37–41 weeks and 6 days), with a birth weight of more than 2500 g. Exclusion criteria were as follows: women who were seropositive for human immunodeficiency virus, or those who had diabetes mellitus, chronic arterial hypertension, acute infection, or haemorrhage with hemodynamic involvement; mothers of premature infants with a low birth weight (<2500 g), twins; neonates with asphyxia; meconium aspiration syndrome; and mothers who did not agree to participate in the study.

The study followed the directives established by the Declaration of Helsinki and all procedures involving patients were approved by the Research Ethics Committees of the University Hospital of Ribeirão Preto and the Women's Health Reference Center of Ribeirão Preto. All subjects gave written informed consent to participate in the study.

Sample collection and preparation

Maternal and UC blood samples were collected into tubes covered with opaque paper in the prepartum and delivery rooms, respectively. The samples were centrifuged and the obtained sera were separated and frozen at −70°C until the time of laboratory analysis.

On the day after delivery and before discharge of the mother–child dyad from the maternity hospital, approximately 1 to 2 mL of colostrum was collected by manual expression into tubes that were protected from light. The samples were immediately frozen at −70°C until the time of later analysis.

Sample analysis

Retinol concentrations were determined by high performance liquid chromatography (HPLC) according to the procedures for serum samples proposed by Arnaud et al.[15] and by Giuliano et al.[16]. Procedures were adapted as described by Ribeiro et al.[17] for HM samples. Retinol concentrations ≤0.70 μmol/L in serum and ≤1.05 μmol/L in milk were considered insufficient.[18]

For laboratory analyses, a Shimadzu LC-20AT model (Shimadzu Cooperation, Tokyo, Japan) was used, with the C-18 type of column (250 × 4.6 mm – 5 μm) and a UV-visible detector (model SPD-20). The mobile phase was composed of acetonitrile:dichloromethane:methanol (proportions, 7:2:1) and the flow rate was 1.0 mL/min. The wavelength used for detecting retinol was 352 nm. Retinol concentrations were determined using the Sigma® external standard (Sigma-Aldrich, St Louis, MO, USA). Prior to the analyses, a standard curve was constructed with known retinol concentrations.

Serum samples (mother's blood and UC blood) were prepared to undergo the extraction procedure as follows. Samples were thawed and homogenized on a Vortex shaker. A volume of 200 μL of serum was pipetted and transferred to another tube. A volume of 400 μL of ethanol was then added and the mixture was vortexed again. A total of 400 μL of hexane was added to this solution and the contents were vortexed for 1 minute. Thereafter, the tubes were centrifuged for 10 minutes at 1600 × g. After centrifugation, a 200-μL aliquot of the hexane (upper) phase was pipetted and transferred to another tube. This content was dried in nitrogen gas flow and suspended in 200 μL of mobile phase (composed of 70% acetonitrile, 20% dichloromethane, and 10% methanol), with subsequent homogenization on a Vortex shaker. The contents were transferred to an HPLC vial in which 20 μL were injected for analysis.

The samples of BM (colostrum) were thawed and the contents of each sample were quantified. A total of 2 mL of 50% KOH and 1 mL of 95% ethanol were added to each 1 mL of sample. The tubes were vortexed for 1 minute and saponified in a water bath at 45°C for 2 hours (saponification releases retinol from retinyl esters, thus allowing the total amount of retinol in the sample to be measured). The samples were then cooled, and 2 mL of hexane was added and the solution was stirred for 1 minute. The contents were centrifuged for 10 minutes at 1600 × g. The supernatant was removed and transferred to another tube. This process was repeated three times and the contents were withdrawn in the same tube. A total of 3 mL were removed from this content, which was dried in nitrogen gas at 37°C and the tube was frozen until the time of HPLC analysis. At the time of analysis, 1 mL of mobile phase was added, and then the solution was homogenized for further transfer into an HPLC vial.

Dietary and other information

Obstetric, socioeconomic, and demographic data, as well as supplement intake data during pregnancy, were obtained by interviewing the mother. VA intake by the mother was determined by applying a quantitative questionnaire of VA intake. A food source questionnaire, which contained a list of 32 food sources of VA adapted from that validated by the International Vitamin A Consultative Group,[19] was used. The frequency and size of the portions consumed during the previous month (last month of pregnancy) were reported with the aid of an album containing photographs of small, medium, and large portions of these foods.[20] Mean daily intake was calculated individually for each parturient using Microsoft Excel 2010 software (Microsoft Corporation, Redmond, WA, USA). This intake was calculated according to the portion size consumed as indicated in the photograph album and the frequency of reported intake, using a table of food composition.[21] When the participants reported intake of vitamin supplements containing VA during the analysis period, the quantity of VA provided by the supplement was added to the amount obtained from the foods consumed. The daily VA intake recommended for adult pregnant women by the estimated average requirement is 550 μg retinol activity equivalents (RAE).[1] RAE intakes of <550 μg were considered inadequate.

Data of the mothers' weight and height and newborns' weight and length were obtained from the medical records, and pregestational weight was reported by the mother. The mothers' weight and height at arrival to the maternity hospital were used to calculate the current body mass index (BMI). This result was used to classify the nutritional status of the pregnant

women according to BMI per gestational age, as recommended by the Brazilian Health Ministry.[22]

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA), with the level of significance set at <0.05 in all analyses. The Student's t-test was used to compare mean retinol concentrations in the blood/UC of the newborns and in BM between the groups with and without adequate serum retinol concentrations. Spearman correlation coefficients were calculated to correlate retinol concentrations in the mothers' blood, in newborns, and in BM, as well as VA intake by the mother. Partial correlation and linear regression analyses using simple and multiple models that were adjusted for age, BMI and VA intake, when needed, were also calculated. Estimates of the parameters and their respective 95% confidence intervals (95% CIs) were obtained for the linear regression models.

The clustering technique and the statistical K-cluster test were used to identify associations between variables. This analysis is characterized by comparison of distinct groups (clustering) consisting of individuals of the population under study who possess similar characteristics regarding some variables of the study.[23,24] In K-cluster analysis, the variables used to form the various groups needed to show a statistically significant difference between groups ($p < 0.05$). Analysis of variance was used to determine the difference between groups obtained by clustering.

Results

General characteristics

Initially, 180 mothers agreed to participate in the study, and a blood sample was obtained from them at the time of admission to the maternity hospital. UC samples were obtained from 159 newborns (88.3%). UC collection was not possible in 21 newborns because of insufficient material. Similarly, colostrum was obtained from 154 (85.6%) mothers, and collection was impossible for 26 because of difficulty in obtaining sufficient material for laboratory analysis. The questionnaire on VA intake could not be applied to one mother because of her refusal to respond. Therefore, complete data regarding the main variables of the study were obtained for 134 (74.4%) participants.

Table 1 shows the sociodemographic, anthropometric, and obstetric characteristics of the population. The age of pregnant women ranged from 18 to 39 years, 61.7% (111/180) were white, and 67.8% (122/180) lived with a partner. The gestational age was approximately 40 weeks Most deliveries were by the vaginal route and 36.1% (65/180) were caesarean deliveries. At admission to the maternity hospital, the BMI of pregnant women was adequate in 35.7%, 14% had a low weight, and 50.3% had excess weight (28.1% were overweight and 22.2% were obese). On average, the pregnant women gained 14.3 kg during pregnancy and over half of them (53.3%) gained more weight than recommended.

Maternal and newborn VA status

The mean retinol concentrations in maternal blood, newborn blood, and BM are shown in Table 2. Mean VA intake by the mothers was 1041.33 ± 1187.86 μg RAE. Insufficient retinol concentrations were detected in more than half of maternal blood samples, in most of the newborns, and in approximately 30% of BM samples. Almost half the mothers had a lower VA intake than recommended (Table 2).

The mean retinol concentration in UC blood of neonates born to mothers with

Table 1. Sociodemographic, anthropometric, and obstetric characteristics of mothers and their newborns at a Brazilian public maternity hospital (Ribeirão Preto, São Paulo, Brazil) (n = 180).

Variables	Mean	±standard deviation
Age (years)	25.7	5.4
Gestational age (weeks)	39.7	1.2
Monthly family income ($US)	879.34	504.39
Monthly *per capita* income ($US)	197.8	104.1
Pregestational body mass index (kg/m^2)	24.4	5.4
Birth weight (g)	3.304	0.4
Length (cm)	49.4	2.1
	n	Percentage
Multiparous	111	61.7
Without a partner	58	32.2
Up to high school	173	96.1
White race	111	61.7
Six or more prenatal visits	160	87.9
Normal delivery	115	63.9
Male newborns	95	52.8
Adequate for gestational age newborns	156	86.7

Table 2. Mean retinol concentrations in body fluids and VA intake, and the prevalence of inadequate (insufficient) retinol concentrations in mothers and newborns at a public Brazilian maternity hospital (Ribeirão Preto, São Paulo, Brazil) (n = 180).

Variables	n	Mean (± SD)	Range	Inadequacy (%)	95% CI
Maternal serum retinol (μmol/L)	180	0.65 (±0.27)	0.12–1.58	103 (57.2)[a]	49.64–64.55
Umbilical cord blood retinol (μmol/L)	159	0.36 (±0.18)	0.07–0.94	151 (94.9)[a]	90.32–97.80
BM retinol (μmol/L)	154	2.95 (±2.70)	0.08–10.63	43 (27.9)[b]	21.00–35.71
VA intake (μg RAE)	179	1041.33 (±1187.86) (61% VA preformed and 39% provitamin A)	50.93–7205.08	80 (44.7)[c]	34.80–54.80

[a]Serum retinol concentrations ≤0.70 μmol/L.
[b]Retinol concentrations in BM ≤1.05 μmol/L.
[c]Total intake < 550 μg RAE/day.
CI, confidence interval; BM, breast milk; VA, vitamin A; RAE, retinol activity equivalents.

insufficient serum retinol concentrations (0.33 ± 0.15 μmol/L) was significantly lower than that in UC blood of neonates born to mothers with adequate retinol concentrations (0.39 ± 0.20 μmol/L, $p = 0.03$). However, there was no significant difference in mean retinol concentrations in BM between these two groups (2.87 ± 2.61 vs 3.07 ± 2.85 μmol/L, $p = 0.64$).

Maternal and newborn VA correlations

There was no correlation between retinol concentrations in maternal blood and in

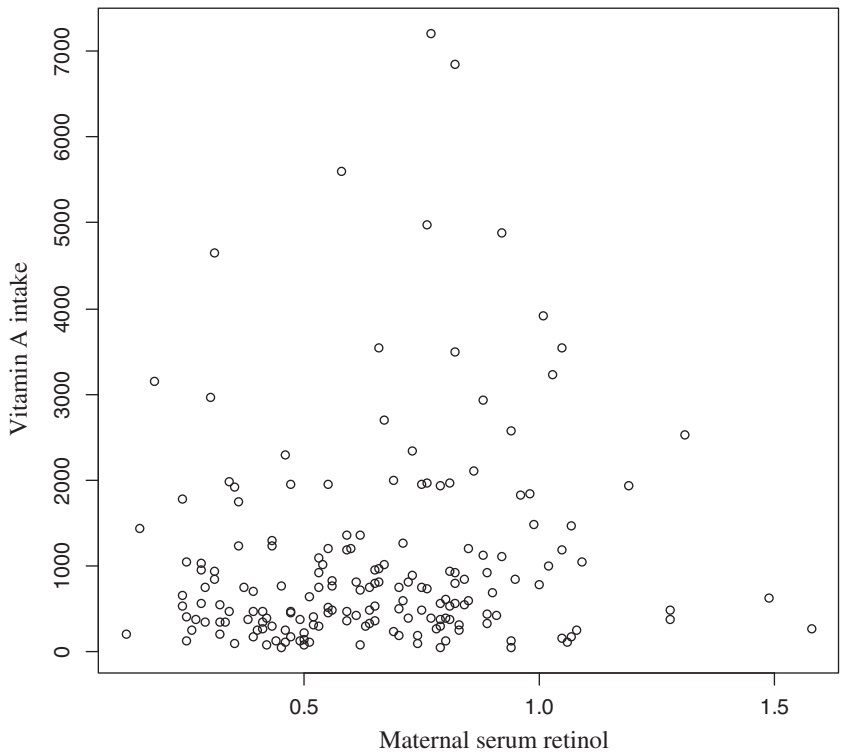

Figure 1. Scatter plot of maternal serum retinol concentrations correlated with maternal vitamin A intake in mothers of a Brazilian public maternity hospital (Ribeirão Preto, São Paulo, Brazil) (n = 180).

UC blood ($r_s = 0.15$; $p = 0.07$), between those in maternal blood and BM ($r_s = 0.02$; $p = 0.76$), or between those in BM and UC blood ($r_s = -0.13$; $p = 0.12$). There were no correlations between VA intake by the mother and retinol concentrations in BM ($r_s = 0.09$; $p = 0.27$) and in UC blood ($r_s = 0.12$; $p = 0.12$). A weak, but significant, positive correlation was observed between VA intake by the mother and retinol concentrations in maternal blood ($r = 0.15$; $p = 0.04$) (Figure 1).

Partial correlation analysis considering the mother's age, maternal VA intake, and BMI showed no correlation between retinol concentrations in maternal blood and newborn blood or between retinol concentrations in maternal blood and BM. A weak, but significant, positive correlation was observed between BM retinol concentrations and newborn retinol concentrations ($r = 0.21$; $p = 0.018$). Partial correlation analyses between maternal VA intake and retinol concentrations in biological fluids (maternal and UC blood, and BM) after adjustment for maternal age and BMI did not show any significant correlations.

Multiple linear regression analyses with adjustment for maternal age, VA intake, and BMI did not show an association between retinol concentrations in maternal blood and UC blood (estimate = 0.09; 95% CI = −0.007–0.2; $R^2 = 0.08$; $p = 0.07$), or between those in maternal blood and BM (estimate = −0.76; 95% CI = −2.43–0.91; $R^2 = 0.07$; $p = 0.36$). No association was observed between maternal VA intake and retinol concentrations in maternal blood,

BM, or UC blood when the data were analysed by multiple linear regression with adjustment for maternal age and BMI.

Analysis by clustering of the variables yielded two distinct groups regarding retinol concentrations in maternal blood and UC blood. Cluster 1 consisted of mothers and newborns with higher values of retinol in maternal and UC blood ($n = 79$). Cluster 2 consisted of mothers and newborns with lower values of retinol in maternal and UC blood ($n = 80$). VA intake by mothers of clusters 1 and 2 was 714.3 and 480.5 μg RAE, respectively ($p = 0.014$).

Discussion

The mean maternal serum retinol concentration observed in this study was 0.65 ± 0.27 μmol/L. This value is below the cut-off point proposed by the World Health Organization for values acceptable as adequate (0.70 μmol/L). This value is similar to those detected in studies conducted on Indian,[25] Albanian[26] and Sudanese[27] women, but are lower than those detected in studies conducted in Germany,[12] Turkey,[28] the United Kingdom,[29] Israel,[30] Czech Republic,[31] and China.[32] More than half of the mothers in the present study had insufficient serum retinol values. This finding may be attributed, in part, to the physiological changes in pregnancy due to accelerated foetal development and to the physiological increase in maternal blood volume during this period.[33] Moreover, another factor that may explain this finding is the low VA intake by these women during pregnancy because half of them ingested less than the recommended amount of VA. Additionally, the mothers in our study belonged to a less privileged economic class, which is more vulnerable to conditions, such as infections, that might increase VA requirements.

With regard to newborns in our study, the mean retinol concentration observed in UC blood was much lower than that observed in maternal blood, which is similar to other studies.[12,26,27,29–32,34–37] Despite these observations, there is currently no agreement in the scientific literature regarding the value of the cut-off point for adequate retinol concentration for newborns, or whether adult values should be chosen for this age range. Low retinol concentrations in newborns may be attributed to regulation of VA transfer through the placenta because high concentrations may have adverse effects on the foetus.[36,38,39] Additionally, the high prevalence of mothers with insufficient serum retinol concentrations could contribute to an explanation of this finding. This is because neonates of mothers with deficient retinol concentrations showed significantly lower retinol concentrations in UC blood compared with those born to mothers without inadequate serum retinol concentrations. Furthermore, newborns normally have reduced retinol concentrations in the liver, with stores increasing during the first months of life, especially when HM contains adequate VA concentrations.[2] In the United Kingdom, Scaife et al.[29] studied mother–child dyads at the time of delivery and found similar findings to the present study.

In the present study, we observed that slightly more than one quarter of the mothers had insufficient retinol concentrations in BM, although the mean level was 2.95 ± 2.70 μmol/L. The wide variability of the observed values may help explain this finding. One of the explanations for this variability is that, because of difficulties inherent to this period of implantation of breastfeeding, collection of colostrum was not standardized. This variability included the breast to be milked, the time of day for the procedure, the time elapsed since the last feeding, whether the milk collected was from the beginning or the end of the feeding, and whether the mother had been feeding recently. These factors might

represent a limitation of this study. Several investigations have detected significant differences in retinol content between the milk obtained at the beginning of feeding and that obtained at the end.[40] Additionally, retinol concentrations in colostrum vary between the fasting and postprandial condition of the mother, being higher after intake of food.[9,41] Furthermore, the low intake of VA-rich foods observed in our study in almost half of the mothers may help explain the high proportion of insufficient retinol concentrations in BM, although no association was detected between these two variables.

In a systematic review, Oliveira-Menegozzo et al.[42] found 14 studies of retinol concentrations in HM, and only colostrum was used in six of these studies. In these studies, when the group of preterm infants was excluded, mean retinol concentrations ranged from 2.48 to 4.50 μmol/L. Among all of the indicators of VA status assessed in the present study, BM showed the highest mean retinol concentration and the lowest proportion of retinol concentrations considered insufficient. According to O'Byrne et al.,[43] the mammary tissue is able to maintain retinoid concentrations in milk for use by the newborn, even in the presence of situations that strongly affect the availability of retinoid in other tissues.

Although the mean VA intake by pregnant women in our study was higher than recommended, its values widely varied. This phenomenon may explain why a VA intake in 44.7% of the pregnant women was below recommended values and why 57.0% of them had insufficient retinol concentrations in blood. Few studies have investigated VA intake in the immediate postpartum period, which may be explained by the difficulty inherent to data collection at this time. Da Silva et al.[44] used a food frequency questionnaire (FFQ) and photographic recording of foods to investigate VA intake in 86 pregnant women at the end of pregnancy. They found a high prevalence of inadequate VA intake, although the mean value was above recommended levels. The authors detected a mean intake of 1490 ± 1283.2 μg RAE/day and observed that 22.1% of women had a lower VA intake than recommended for this phase of life. Lee et al.[45] studied the prevalence of VA in pregnant women in Bangladesh. They observed that 53% of them had a lower intake than the recommended amount of VA as assessed with a semi-quantitative, 24-hour, food recall questionnaire. Scaife et al.[29] investigated pregnant women in the 34th week of gestation using a semi-quantitative FFQ. They found that mean VA intake was below the reference value and that 69% of the subjects ingested lower than recommended amounts. In India, Basu et al.[46] performed a study of food intake by pregnant women in the 7th month of gestation using a 24-hour recall and an FFQ. They observed that 75.2% of the subjects had ingested less than 25% of the recommended amount of VA. These authors also detected wide variations in VA intake among subjects, as also observed in the present study.

The wide variability of VA intake by mothers in our study could be explained by several factors. One factor is the presence of this micronutrient in specific foods that are normally not consumed on a daily basis.[1] A second factor is changes imposed by pregnancy on the organism, including those related to eating habits, which may be modified by taboos, beliefs, and physiological changes, such as nausea, heartburn, and gastroesophageal reflux.[47] A third factor is questions inherent to the method of measurement (questionnaire) of this variable, which depends on the memory of the person interviewed and is likely to be biased.[48] A fourth factor is the time when these data were collected (i.e., the immediate postpartum period, which has biopsychosocial implications and limitations for women who have recently given birth).[49]

Additionally, there is no valid FFQ to estimate the intake of VA by pregnant women, and this is another limitation of this study.

Almost half of the mothers in our study had a lower VA intake than recommended. However, only 14% of them were underweight and half of them were overweight in relation to their gestational age at the end of pregnancy. This observation suggests that the inadequate intake of VA was not due to an insufficient amount of food, but rather to a low density of this micronutrient in foods that were preferentially consumed. Inadequate VA intake is high in all regions of Brazil, reflecting the poor quality of the diet of Brazilian people. Data of the National Dietary Survey of the latest Family Budget Survey showed that 72% of Brazilian women of childbearing age consume inadequate amounts of VA.[50,51] Mothers in the current study had a low income and low educational level. A lack of financial resources and difficulty in processing information may prevent access of economically underprivileged classes to a balanced diet, with the consequent higher risk of nutritional deficiencies, such as low retinol concentrations.[52–54]

Retinol and its metabolites may inhibit adipogenesis, enhance apoptosis of fat cells, and regulate the synthesis of several adipokines.[55] Some studies have also shown that VAD is associated with obesity, especially in severe cases.[56,57] In the present study, 22% of the mothers were obese. However, there were no differences in the mean retinol intake and retinol concentrations in maternal milk, as well as mean serum retinol concentrations in maternal and UC blood in the different nutritional status groups of the studied mothers. A lack of VA-rich food intake in the diet in the majority of mothers in this population could explain this phenomenon. Similarly, obese mothers may have a high intake of high-calorie foods, but at the same time, have low intake of micronutrients, such as VA.

Our study differs from previous studies with Brazilian mothers because we analysed VA concentrations in three body fluids, in addition to studying vitamin intake. Dos Santos et al.[11] studied only maternal intake of VA. Ramalho et al.[37] studied VA concentrations in two fluids (mother's serum and UC blood) and Da Silva et al.[44] studied two body fluids (mother's serum and breast milk) and maternal ingestion. Dos Santos et al.[11] studied 322 Brazilian pregnant women and found inadequate intake of VA in 71% of them, which is higher than that found in the present study (44.7%). Ramalho et al.[37] studied 291 postpartum women and their newborns. They found that 22% of postpartum women and 54.2% of newborns had inadequate serum retinol concentrations, which were lower than those found in our study (57.2% and 94.9%, respectively). Da Silva et al.[44] studied 86 healthy mothers who were recruited within 16 hours after delivery. They found that 36.7% of the mothers had inadequate intake of VA (lower than that found in the present study), and 9.3% and 22.1% had inadequate VA concentrations in serum and in BM, respectively. These rates are also lower than those of our study.

Some studies have analysed the correlation between retinol concentrations in the mother's blood and UC blood, but varied results have been found. A study in Zimbabwe[58] ($r = 0.73$) and another study[59] in Brazil ($r = 0.8$) showed a strong positive correlation between retinol concentrations in the mother's blood and UC blood. Saunders et al.[60] observed a significant association between retinol concentrations in maternal blood and UC blood of mothers from a maternity hospital in Rio de Janeiro ($\chi^2 = 21.53$). Previous studies in Israel[30] ($r = 0.29$), India[34] ($r = 0.27$), and Brazil[61] ($r = 0.27$) showed a weak positive

correlation between these variables. A previous study[32] in Chinese mothers did not find such a correlation.

Few previous studies have evaluated the correlation between maternal serum retinol concentrations and retinol concentrations in colostrum or VA intake. De Lira et al.[62] studied 103 infants in a public maternity hospital. In addition to showing similar average concentrations of retinol in colostrum to those of the present study, they also did not find a correlation between serum retinol concentrations of the mother and retinol concentrations in colostrum. Da Silva et al.[44] studied 86 healthy parturients who were recruited within 16 hours postpartum. They found no linear correlations between serum retinol concentrations, retinol concentrations in colostrum, and VA intake. In our study, these variables studied showed at most poor correlations.

We used the same method (HPLC) of a single micronutrient (retinol) in different body fluids (UC, blood and BM), which could theoretically have some correlation with each other. However, we did not observe any significant positive associations of retinol concentrations among the various fluids. However, a positive association was observed when we grouped metabolic variables (K-cluster). Mothers who had lower VA intake also had lower serum retinol concentrations, as also found in the UC blood of their newborns. No correlation was observed between maternal VA intake and maternal serum retinol concentrations in partial correlation analysis after adjustment for age and BMI. The association detected by the K-cluster method is explained by the fact that statistical aggregation of subjects with similar results divided the samples into two opposite metabolic groups regarding VA values in maternal serum and in the UC. Two distinct metabolic groups showed significantly different VA intakes. Partial linear Spearman correlations and linear regression tests did not show any important statistical results, probably because many biological associations are usually not linear. Therefore, the metabolic mechanism is still incompletely understood (homeostatic control of retinol concentrations in maternal blood, placental barrier against the passage of VA from mother to foetus, VA transport from maternal blood to the mammary glands, among others). Additionally, small differences in collection and in the method of determination in the different fluids may help explain the lack of a correlation between these biochemical variables. However, the lack of finding a correlation between VA intake and retinol concentrations in the fluids in our study may also be explained by the high variability in intake of retinol-rich foods, by the difficulty inherent to the time of application of the questionnaire (immediate postpartum period), and by the memory biases characteristic of this method.

According to the National Survey of Demography and Health of Children and Women (PNDS-2006)[14] conducted by the Brazilian Ministry of Health, 16.1% of Brazilian women of reproductive age were between 15 and 19 years of age. Moreover, at the time of the research, almost a quarter (24.6%) of births occurred in women younger than 20 years old. In our study, by ethical and legal criteria, we only included women older than 18 years of age (legal age of majority in Brazil). Additionally, mothers who gave birth to children weighing less than 2500 g were not included in our study. According to the PNDS-2006, 12.5% of women of reproductive age had incomplete and/or complete university degrees, while in our population, only 3.9% had such a level of education. Finally, the highest proportion of women who gave birth in the 5 years prior to the survey was self-described as brown or black, while in our study, 61.7% were white. To corroborate such findings,

in 2016, the Brazilian Institute of Geography and Statistics presented the document "Synthesis of social indicators: an analysis of the living conditions of the Brazilian population".[63] This document includes data obtained from the National Household Sample Survey - PNAD 2015, which covers the entire national territory (not just women). When we compared data from the population of our study with this national survey, we observed large differences. The percentage of the Brazilian adult population with a higher education was 14.7%, while in our study, it was only 2.2%. The average income was R$1,270 in this previous survey, while the income in our study was only R$645. Finally, in the national study, more than half (53.9%) of the people declared themselves as black or brown, while the percentage of those who declared black or brown in our study was 38.3%.[63] The characteristics of the studied population reflect the fact that our study was carried out in a public maternity hospital, which predominantly has women from the less privileged socioeconomic classes. Therefore, importantly, the present sample was not representative of the general Brazilian population of mothers in the immediate postpartum period and their newborns. This is another limitation of our study.

Therefore, further studies are required to understand the relationship between VA intake and dynamics in different body fluids, especially during pregnancy and breastfeeding. More studies are also required to determine the real dimension of VAD during times of a high prevalence of obesity, especially in less economically privileged communities in developing countries.

In conclusion, the present study shows a high proportion of insufficient retinol concentrations in the UC, maternal blood, and BM at the largest public maternity hospital in Ribeirão Preto (São Paulo, Brazil). There is also a high percentage of pregnant women with inadequate VA intake during the month preceding delivery. Mothers with insufficient mean serum retinol concentrations delivered neonates with lower concentrations of this micronutrient in UC blood. Additionally, mothers who had lower VA intake also had lower serum retinol concentrations, as observed in the blood of their newborns.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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