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## Composition of breast milk of lactating adolescents in function of time of lactation

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### Abstract

The aim of this study was to evaluate the composition of breast milk of lactating adolescents in function of lactation time.

**Methods:** We followed 51 lactating adolescents, between the 6<sup>th</sup> and 14<sup>th</sup> weeks postpartum (WPP). The determination of fat, protein and lactose in milk were conducted, respectively, by the methods of Lucas, Lowry and Perry & Doan. Micronutrients were determined by atomic absorption spectrophotometry. Data was presented by the mean and standard error. ANOVA with repeated measures was used and Tukey as post test. It was accepted a significance level of 5%.

**Results:** There was a significant reduction ( $P < 0.05$ ) in protein content during the postpartum weeks studied (6<sup>th</sup>:  $16.6 \pm 1.1$ ; 10<sup>th</sup>:  $13.7 \pm 1.0$ ; 14<sup>th</sup> WPP:  $12.3 \pm 1.1$  g/day). The lactose (6<sup>th</sup>:  $60.2 \pm 1.9$ ; 10<sup>th</sup>:  $60.4 \pm 2.6$ ; 14<sup>th</sup> WPP:  $65.1 \pm 4.0$  g/day) and fat (6<sup>th</sup>:  $41.6 \pm 3.3$ ; 10<sup>th</sup>:  $36.2 \pm 3.4$ ; 14<sup>th</sup> WPP:  $31.5 \pm 9.0$  g/day) concentration remained unaffected. The zinc concentration in the breast milk was lower than that is commonly found in literature (mean 1.16 mg/day). The copper, iron, calcium and phosphorus concentration was sufficient to meet the needs of the infants between 0 and 6 months old.

**Conclusion:** The lactation period did not influence the concentration of micronutrients, lactose and lipids, but there is a reduction on protein of the breast milk. In spite of the reducing concentration of protein, it is sufficient to meet the needs of infants from 0 to 6 months old.

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Key words: Lactation. Adolescence. Weeks postpartum. Nutrients.

### COMPOSICIÓN DE LA LECHE DE MADRES LACTANTES ADOLESCENTES EN RELACIÓN CON EL TIEMPO DE LACTANCIA

#### Resumen

El objetivo de este estudio fue evaluar la composición de la leche materna de lactantes adolescentes en función del tiempo de la lactancia.

**Métodos:** Se siguieron 51 adolescentes lactantes, entre la semana 6 y 14 después del parto (WPP). Se llevaron a cabo la determinación de grasa, proteína y lactosa de la leche, respectivamente, por los métodos de Lucas, Lowry y Perry & Doan. Los micronutrientes se determinaron por espectrofotometría de absorción atómica. Los datos fueron presentados por la media y el error estándar. ANOVA con medidas repetidas se utilizó Tukey como prueba post. Se aceptó un nivel de significación del 5%.

**Resultados:** Se observó una reducción significativa ( $P < 0,05$ ) en el contenido de proteína en las semanas después del parto estudiados (sexto:  $16,6 \pm 1,1$ ; 10 a:  $13,7 \pm 1,0$ ; 14a SPP:  $12,3 \pm 1,1$  g/day). La lactosa (sexto:  $60,2 \pm 1,9$ ; 10º:  $60,4 \pm 2,6$ ; 14 a SPP:  $65,1 \pm 4,0$  g/day) y grasa (sexto:  $41,6 \pm 3,3$ ; 10º:  $36,2 \pm 3,4$ ; 14 a SPP:  $31,5 \pm 9,0$  g/day) concentración no se vio afectado. La concentración de zinc en la leche materna fue menor que se encuentra comúnmente en la literatura (media de 1,16 mg / día). La concentración de cobre, hierro, calcio y fósforo es suficiente para satisfacer las necesidades de los niños entre 0 y 6 meses de edad.

**Conclusión:** El período de lactancia no influyó en la concentración de micronutrientes, la lactosa y los lípidos, pero hay una reducción en la proteína de la leche materna. A pesar de la reducción de la concentración de proteína, que es suficiente para satisfacer las necesidades de los bebés de 0 a 6 meses de edad.

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Palabras clave: Lactancia. Adolescencia. Semanas después del parto. Nutrientes.

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## Abbreviation list

- WPP: Weeks postpartum.  
UFRJ: Federal University of the Rio de Janeiro State.  
BMI: Body Mass Index.  
WHO: World Health Organization.  
SPSS: Statistical Package for the Social Sciences.  
SISNEP: National Information System Ethics in human Research.  
Kg: Kilogram.  
Cm: Centimeter.  
IOM: Institute of Medicine.  
FAO: Food and Agriculture Organization.  
RV: Reference value.  
FAPERJ: Research Foundation of the State of Rio de Janeiro.  
PROPI: Pro Rectory of Research, Graduate Studies and Innovation of the Federal Fluminense University.  
CNPq: National Research Council.

## Introduction

Pregnancy and lactation are anabolic processes controlled by hormones that direct the nutrients to highly specialized maternal tissues such as the placenta and mammary gland, and promote its transfer to the developing fetus and neonate<sup>1</sup>. Adolescents require nutrients for their own growth and physical development. The needs required for pregnancy, fetal growth and lactation is added to it. Therefore, when pregnancy and lactation occur in teenagers these needs are superimposed on adolescence<sup>2</sup>.

It is known that human milk is the first food for most newborns, being the main source of all nutrients needed to satisfy the biological functions, promoting growth and development during the first months of life<sup>3</sup>. Therefore, lactation during adolescence is an aspect that has special importance in reducing the potential risk that the child suffers in the first year of life.

Despite the large number of teenage mothers observed, worldwide studies on the composition of their milk are still quite conflicting and in Brazil there are few studies on the composition of breast milk of adolescents in different weeks postpartum (WPP)<sup>4,5</sup>.

The relevance of this study is related to the high number of teenage pregnancy, especially in the low-income population of the Brazilian urban centers and consequently high number of teenage mothers. Thus, this study aims to assess the breast milk composition of the lactating adolescents, in function of time of lactation.

## Methodology

This is a longitudinal observational and analytical study where lactating adolescents were followed

between 6<sup>th</sup> and 14<sup>th</sup> weeks postpartum (WPP). The following criteria for inclusion in the study were used: exclusive breastfeeding (breast milk with no supplementation of water or tea) or predominant (with inclusion of water or tea)<sup>6</sup>, less than 18 years old, without infectious disease, no use of nutritional supplements and medications that could interfere with the interpretation and analysis of results. The study was conducted in the year 2011.

This was a convenience of random sample, where all lactating adolescent enrolled in the Polyclinic of Specialties in the Health Care of Women Malu Sampaio, in the Niterói city, and in the Hospital Maternity of the Federal University of Rio de Janeiro (UFRJ), among december 2011 to december 2012, were invited to participate in the study. However, only 51 adolescents agreed to participate. The voluntary writing for the participation in the study was obtained after explaining the purpose of the study and use of the data under the guarantee of anonymity.

The body mass index (BMI)/age was used to evaluate the nutritional status of voluntary, based on criteria established by WHO, 2007<sup>7</sup>: considering obesity the BMI/age > 97th percentile, overweight the BMI/age between 85th and 97th percentile, eutrophic between 3<sup>th</sup> and 85th and underweight BMI/age below the 3th percentile.

Samples of mature breast milk (20 ml) were obtained by manual extraction in sterilized *Falcon* tubes, three different times: 1) at 6 weeks, 2) at 10 weeks and 3) in the 14<sup>th</sup> WPP. Samples of milk were obtained from breast that had not been sucked in the last breastfeeding. These samples were collected in the morning between 9 and 10 hours. After the collection, milk was used to determine creamatocrit<sup>8</sup>, immediately. Other milk aliquots were separated in *ependorfs* tubes for subsequent analysis of proteins, lactose and minerals. For the determination of protein concentration the *Lowry*<sup>9</sup> method was used and lactose was determined by the Perry and Doan<sup>10</sup> method. The concentration of macronutrient was expressed in g%. The minerals iron, copper and phosphorus were determined by atomic absorption spectrophotometry using the apparatus Varian (Australia) model AA240Z; for minerals calcium and zinc the device Varian (Australia) model AA240FS.

Data was presented by the descriptive statistic such as mean and standard error. ANOVA with repeated measures was used for means of comparison between the three periods studied and *Tukey* used as posttest. The significance level of 5% was accepted. The Statistical Package for the Social Sciences (SPSS 2005) for Windows, version 14.0 was used for analyzes. The assumption of normality (Gaussian distribution) of the data was checked using the tests of *Skewness* and *Kurtosis* to support the use of the statistical methods described above.

The study protocol complies with the ethical principles contained in the Declaration of Helsinki and the rules of

Resolution 196/96 of the National Health Council and was approved by the Ethic Committee in Humans Research of the Municipal Health and Civil Defense of the Rio de Janeiro city, consistent with the National Information System Ethics in human Research (SISNEP) protocol number 0371.0.258.314-11 and of the Antonio Pedro University Hospital of the Fluminense Federal University under protocol number 070/06.

## Results

The general characteristics of the lactating adolescents and of their infants are shown in table I. It can be observed that the average age of the adolescents were 16 years, with menarche at 12, first sexual intercourse at 14 years of age, and gynecological age of 4 years old (defined as the chronological age at delivery minus the age of menarche). Most adolescents (95%) had adequate number of prenatal visits (> 6) and satisfactory gestational weight gain. The weight, length and head circumference of the infants were normal at birth. However, 7.14% presented body weight less than 2.500 g, being small for gestational age.

The anthropometrical measures and the BMI of adolescents, throughout the study, are shown in table II. It can be observed a significant increase of the body weight and BMI of the mothers during the study when compared with the pre gestational period. The nutritional status classification showed a higher frequency of lactating adolescents with normal weight (72.7%) followed by overweight (22.7%) and only 4.6% with low weight.

**Table I**

*Reproductive, anthropometric and general characteristics of the lactating adolescents and their infants (n = 51)*

Characteristics	Mean ± SE
Mother Age (years)	16.37 ± 0.15
Menarche (years)	12.08 ± 0.14
First sexual intercourse (years)	14.37 ± 0.17
Gynecological age (years)	4.28 ± 0.18
Gestational weight gain (kg)	13.86 ± 0.67
Weight at birth (g)	3181 ± 88
Length at birth (cm)	48.3 ± 0.37
Head circumference at birth (cm)	33.8 ± 0.29

**Table II**

*Pre gestational nutritional status and in different weeks postpartum of the lactating adolescents*

Indicator	Pre gestational	6 <sup>th</sup> WPP	10 <sup>th</sup> WPP	14 <sup>th</sup> WPP
Weight (Kg)	54.3 ± 10.0 <sup>a</sup>	59.7 ± 13.2 <sup>b</sup>	61.6 ± 14.3 <sup>b</sup>	62.7 ± 16.1 <sup>b</sup>
Height (m)	1.6 ± 0.05	1.6 ± 0.05	1.6 ± 0.05	1.61 ± 0.06
BMI/age (kg/m <sup>2</sup> )	21.0 ± 6.0 <sup>a</sup>	23.1 ± 4.1 <sup>b</sup>	23.4 ± 4.2 <sup>b</sup>	23.6 ± 5.0 <sup>b</sup>

Different letters between columns denote statistical difference.

The macronutrient concentration of breast milk of lactating adolescents in different weeks postpartum is presented in table III. It can be observed that significant reduction ( $P < 0.05$ ) in the protein concentration occurred in the 14<sup>th</sup> WPP. The lactose and fat concentration remained unchanged throughout the study. It is important to emphasize that the change in the concentration of protein and total fat, lead to a non-significant reduction in the energy content of breast milk (25%), in the 14<sup>th</sup> WPP.

Table IV shows the concentration of minerals present in the breast milk in different weeks postpartum. It can be observed that the concentration of the minerals did not change significantly during the study. However, the concentrations of zinc were below the values considered appropriate for all weeks postpartum (WPP).

Positive associations were observed between the energy, total fat ( $r = 0.86$ ;  $p < 0.01$ ) and protein content ( $r = 0.72$ ;  $p < 0.01$ ) in the breast milk of lactating adolescents. Positive associations were observed, too, between the content of copper with iron ( $r = 0.37$ ;  $p = 0.02$ ) and zinc ( $r = 0.43$ ;  $p = 0.04$ ), in the 10<sup>th</sup> WPP. When the values of the minerals found in the milk of adolescents were compared with the daily requirement of infants aged 0 to 6 months it was observed that the nutrients concentration were adequate to satisfy the needs of newborns in the different periods of the study, with the exception of zinc.

## Discussion

The lactating adolescent remained with adequate BMI during the study. But, it is important emphasize that they didn't lost weight as recommended for IOM (1991)<sup>12</sup> at the end of the study. Others researchers has been observed similar results in Brazilian lactating adults women<sup>13,14</sup> and adolescents<sup>15</sup>. Lactation is a moment of the reproductive cycle that, actually, is associated with body weight retention in adult women<sup>11</sup>. However, it is questionable whether this increase in body weight, after delivery, may be associated with obesity in adulthood<sup>13,15</sup>. None association was found between nutritional status and milk composition.

There are few published studies about the composition of macronutrients and micronutrients in the milk of lactating adolescents, especially in Brazil. Hence,

**Table III**  
*Energy and macronutrient concentration of the breast milk of lactating adolescents in different weeks postpartum*

Macronutrient and energy	RV	Mean for the period	6 <sup>th</sup> WPP	10 <sup>th</sup> WPP	14 <sup>th</sup> WPP	P-value
Fat (g/day)	31.0	36.4±5.2	41.6±3.3	36.2±3.4	31.5±9.0	0.24
Protein (g/day)	9.1	14.2±1.1	16.6±1.1*	13.7±1.0	12.3±1.1	0.01
Lactose (g/day)	60.0	61.9±2.8	60.2±1.9	60.4±2.6	65.1±4.0	0.27
Energy (kcal/day)	555.0	632.3±62.7	681.6±41.4	622.2±45.1	593.1±101.4	0.07

Reference value (RV) based on Dietary Reference Allowance<sup>19</sup>. Results considering a standard volume of 0.78 L/day of adult human milk secreted per day. Variables with normal distribution (test *Kolmogorov-Smirnof*). \*Significantly different from the 14<sup>th</sup> WPP.

**Table IV**  
*Mineral concentration in breast milk of lactating adolescents in different weeks postpartum*

Minerals	Reference Value*	Mean for the period	6 <sup>th</sup> WPP	10 <sup>th</sup> WPP	14 <sup>th</sup> WPP	P-value
Iron (mg/day)	0.27	0.35 ± 0.1	0.34 ± 0.04	0.37 ± 0.07	0.34 ± 0.07	0.85
Zinc** (mg/day)	2.0	1.16 ± 0.1	1.31 ± 0.1	1.18 ± 0.11	1.00 ± 0.12	0.08
Copper (µg/day)	200.0	220.0 ± 26.7	270.0 ± 30.0	190.0 ± 20.0	200.0 ± 30.0	0.07
Phosphorus (mg/day)	100.0	116.8 ± 5.9	122.3 ± 3.8	115.8 ± 6.5	112.2 ± 7.3	0.21
Calcium (mg/day)	210.0	266.7 ± 15.2	279.5 ± 12.3	263.6 ± 16.9	257.3 ± 16.6	0.36

\* Reference value (RV) based on Adequate Intake (AI)<sup>10</sup>. \*\*Zinc: 4 WPP: 2.15mg/day; 8WPP: 1,56 mg/day; 12 WPP: 1,15 mg/day<sup>10</sup>. Results considering a standard volume of 0.78 L/day of adult human milk secreted per day. Variables with normal distribution (test *Kolmogorov-Smirnof*).

the importance of conducting studies to expand knowledge on this subject.

Some researchers showed that the total content of protein in milk of adult women can change in relation to the time of lactation<sup>16</sup>. The content of about 16.0g/L in colostrum falls to about 9.0 g/L in mature milk<sup>17</sup>. The concentration of proteins found in the present study was higher than these values and in other studies with adult women in different countries<sup>15,16,17,18</sup>, but similar to the value found in a study with lactating adolescents in Brazil<sup>4</sup>. Maybe this higher concentration could be related to different methods used to determine protein concentration in breast milk or due the characteristics of the adolescence.

This study shows a decrease in the protein content of milk from the 10<sup>th</sup> week postpartum. However, remaining at a concentration capable of meeting the needs of children in the first semester of life (9.1 g/day)<sup>19</sup>. It has been proposed that changes in the concentration of nutrients depend on the time of lactation. It may reflect changes in the nutritional needs of the baby due to the decrease in growth rate and also due to the increased capacity of the digestive system of the child<sup>17</sup>.

Lactose is the main carbohydrate in human milk<sup>20</sup>. Its concentration in mature milk of adult women is approximately 75 g/L<sup>21</sup>. The concentration of lactose found in the present study was similar to this value and to others studies with adult women in different countries<sup>17,22,23</sup> and in Brazil<sup>24</sup>; and in the milk of lactating adolescents in Rio de Janeiro<sup>4</sup>. The lactose concentration remained unchanged during the studied period.

These results corroborated the literature data. The concentration of lactose found in the milk of lactating adolescents met the needs of child in the first semester of life (60g/day - IOM, 2002)<sup>19</sup>.

Typically, the concentration of fat in mature milk of adult women is 35 g/L<sup>17</sup> and in general, the amount of milk lipids does not change, but its quality. The concentration of fat found in the 14<sup>th</sup> WPP, in the present study was lower than that found in adult women in Venezuela (43 g/L)<sup>18</sup>, but similar to others reported for adult women from developing countries (31 to 35g/L)<sup>25</sup> and in Brazil<sup>5,24,26</sup>. The concentration of fat found in the milk of adolescents was in accordance with recommendations by the IOM, 2002<sup>19</sup> (31g/day) to meet the needs of infants 0-6 months of life.

In general, the average concentration of protein in breast milk of lactating adolescents in the study presented above the usual values reported in literature for adult women<sup>17,24</sup>; and the lactose and fat content was similar to it. Positive correlations between the energy content of fat and milk protein were found and were expected, since these macronutrients are responsible for the energy supply of milk.

The iron content of breast milk is often characterized as low, but with high bioavailability, above 70%<sup>27</sup>. In accordance with IOM, 2001<sup>31</sup>, the neonate born with adequate endogenous storage and it could supply the needs to hemoglobin and mioglobin production, during six months. In the present study, the iron content (0.45 mg/L) presented similar to that reported by others researchers<sup>4,28,29</sup> in lactating adolescents and adults in the first semester postpartum. The iron concentration in

the milk of adolescents studied remained constant, although other studies have shown a significant reduction of iron in relation to lactation stage<sup>27,29,30</sup>. This difference can be attributed to the fact that those studies evaluated the changes in the iron content in the breast milk from the colostrum to mature milk. The content of iron found in the present study agreed with recommendations by the IOM, 2001<sup>31</sup> (0.27 mg/day) to meet the needs of infants 0-6 months of life.

The zinc concentration in breast milk of lactating adolescents (1.16 mg/day) presented below that reported for mature milk in adult women, in different countries<sup>23,28-30,32,33</sup>. But similar that observed by Mastroeni et al (2006)<sup>30</sup> in mature milk of adult women (1.17 mg/day) from São Paulo, in the 8<sup>th</sup> WPP. Despite its concentration was constant during the studied period, the zinc concentration presented near the recommendation of FAO, 2001 (1.1 mg/day)<sup>34</sup> until 10<sup>th</sup> WPP, but below to IOM recommendation (2.0 mg/day)<sup>31</sup>. However, it may be consider that the neonate born with liver zinc storage that is capable to complement his need and maintain adequate zinc level in blood<sup>31</sup>. In the present study, it does not appear to be related to the anthropometric characteristics of the volunteers studied.

In relation to copper in the milk of adolescents, it showed similar to that reported by Silvestre et al., 2000<sup>29</sup> and Azeredo, 2005<sup>4</sup>. However, lower than that observed in other studies<sup>23,28,32</sup>, showing that there are conflicting results in the literature. In the present study copper content was in accordance with the recommendation of the IOM, 2001<sup>31</sup> as the amount of this nutrient sufficient to meet the child's needs. But it must be considered that the amount of copper observed in this study is considered the minimum to be ingested by a child. Like iron and zinc, copper atoms are stored in the liver before birth to be utilized during the breastfeeding<sup>31</sup>.

Some studies showed that lactating women secrete approximately 210 mg of calcium per day, in mature breast milk<sup>35</sup>. Some researchers<sup>32</sup> found an increase in the calcium content from colostrum to mature milk in adult women in São Paulo, and suggest this variation occurs to meet the nutritional needs of the child in the first year of life.

The calcium and phosphorus concentration in the present study were similar to that found in lactating adult<sup>4,23,32</sup>. In human milk, a Ca/P ratio about 2.2 is important for maintaining proper bone mineralization of the child<sup>35</sup>. The ratio obtained in this study was similar to that (2.28). In the present study the calcium and phosphorus concentrations in milk were not affected by the stage of lactation, unlike that showed in other studies<sup>4,23</sup>. The content of calcium and phosphorus remained adequate throughout the period studied, according to the recommended for infants by IOM, 2001<sup>35</sup>.

None association were observed between micronutrients content in breastmilk and the maternal characteristics.

## Conclusion

This study suggested that, only, protein milk concentration is influenced by the lactation period and its concentration seems to be higher than that found in adult women. The iron, copper, calcium and phosphorus concentration seems adequate to supply the need of the neonate during exclusive breastfeeding and does not change with the lactation period. But zinc concentration of breast milk is not quite sufficient to satisfy the needs of the child during six months postpartum. The concentration of fat, lactose, iron, copper, calcium and phosphorus of the adolescent breast milk is adequate and quite similar to adult women.

The literature does not present conclusive data on this issue, so more research is needed to expand knowledge about the concentration of micronutrients in breast milk of adolescents. It is important know if there is influence of the concentration of micronutrients in maternal blood on milk.

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