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by Martini Martini

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Breastmilk Macronutrient Levels and Infant Growth During the First Three Months: a Cohort Study

Martini, M.D.*, Irwanto, M.D., Ph.D.*,, Roedi Irawan, M.D., Ph.D.*, Nur Aisiyah Widjaja, M.D.***

**Department of Child Health, Faculty of Medicine, Universitas Airlangga. Jl Mayjen Prof Moestopo 47, Surabaya 60132 East Java-Indonesia,*

*** Universitas Airlangga Hospital, Kampus C Mulyorejo, Surabaya 60115 East Java-Indonesia.*

ABSTRACT

Objective: The first three months after birth is a critical time interval for growth and development. Breastmilk is a natural nutrition source for infants. However, studies on the practice of exclusive breastfeeding and infant growth tend to result in contradictions. The objective of this study was to investigate the correlation between breastmilk macronutrient levels and infant growth during the first three months.

Methods: We conducted an observational cohort study at Universitas Airlangga Hospital from June-October 2018. Subjects were enrolled using total sampling. Infant anthropometry, as defined by body weight, body length, and head circumference, were measured. Breastmilk specimens were collected using a breast pump and then sent directly for analysis. Lactose, protein, fat, and total calorie levels were obtained using a human milk analyzer. Procedures were repeated three times, once per month. The Pearson correlation coefficient was used for statistical analysis.

Results: Forty participants were enrolled in this study. There was a positive correlation between breastmilk total calories and head circumference growth during the first (p = 0.039), second (p = 0.020), and third month (p = 0.020). Breastmilk protein level was positively correlated with body length ($p < 0.05$) and head circumference ($p < 0.05$) during the first month. There was no correlation between body weight and breastmilk macronutrients or total calories ($p > 0.05$).

Conclusion: Breastmilk macronutrient levels correlate to infant growth in a unique pattern. Total calories and first month protein correlated positively with infant head circumference. However, calorie source, e.g., lactose or fat, did not correlate with infant body weight and length.

Keywords: Breastmilk; nutrition; growth; infant; macronutrient (Siriraj Med J 2020; 72: 10-17)

INTRODUCTION

Growth and development are the main features that distinguish between children and adults. The first three months after birth is a critical time interval for growth and development. The brain volume increases quickest during this time, at roughly 1% per day after birth; the rate slows to 0.4% per day by the end of third month.[1] Child growth itself is commonly associated with nutrition intake. Abnormal nutrition intake will result in aberrant growth. In return, deviation from normal growth may indicate serious health problems.[2] Hence, nutrition is an important aspect to be observed during childhood.

Breastmilk is a natural nutrition source for infants. Human breastmilk contains specific components that avert malnutrition and offer optimal growth for infants.[3,4]

Corresponding author: Irwanto

E-mail: irwanto.idris@gmail.com

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ORCID ID: http://orcid.org/0000-0002-7573-8793

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Lactose, a main breastmilk component, is positively correlated to infant weight, adiposity, and body mass index in the 3-month-old infant.[5]

Although various proteins in breastmilk are lower than in formulated milk,[6] breastmilk contains specific free amino acids (such as glutamine) that are positively correlated with length gain.[7,8] Another study showed that a higher n-3/n-6 ratio in breastmilk positively correlates with infant length.[9] However, no matter how good breastmilk may appear to be for the infant, studies on the practice of exclusive breastfeeding and infant growth tend to result in contradictions. According to an Indonesian Health Department report, although exclusive breastfeeding increased from 15.3% to 30.2% between 2010-2013, stunting and malnutrition remain serious problems. One study showed that exclusive 6-month breastfeeding leads to underweight infants.[10] However, other studies instead reported overweightness in exclusively breastfed infants.[9,11] These differences do not apparently correlate with the breastfeeding itself, but rather are more correlated with breastmilk macronutrient content.[11,12] Hence, in this study we aimed to investigate the correlation between breastmilk macronutrients and infant growth during the first three months.

MATERIALS AND METHODS

Study design

This study was an observational cohort deign that aimed to explain the correlation between breastmilk macronutrients, defined by carbohydrates (in the form of lactose), protein, and fat, and infant anthropometry, defined by body weight, body length, and head circumference. This study was conducted at the Universitas Airlangga Hospital from June to October 2018. Ethical clearance was issued by the Ethics Committee at the Universitas Airlangga Hospital, Surabaya, Indonesia, with clearance number 108/KEH/2018.

Study subjects

Subjects were enrolled using total sampling methods, where all candidates who met inclusion criteria were recruited during the study period. Inclusion criteria were term neonates with a birthweight > 2,500 g. The infant's mother must exclusively breastfeed without any additional nutritional intake for at least three months. Exclusive breastfeeding was not defined by a daily measurable dose, but rather as natural as possible where the infant was satiated. The mothers also had to have their Mother and Child Health Handbook still available for data tracking and cross-checking. The Mother and Child Health Handbook is a book officially given by the Indonesian government to each pregnant mother in order to help keep track of her and her baby's health status. Informed consent signed by the mother was required to participate in this study. Preterm infants, infants with congenital disease, and mothers with HIV infection who were unable to breastfeed were excluded from this study. Participants were considered dropped-out when the mother did not exclusively breastfeed, the Mother and Child Health Handbook was missing, the mother or infant did not survive during our observation, or she decided to withdraw from the study.

Study questionnaire

A questionnaire was provided to obtain characteristics about the mothers and infants, as well as a tool for monitoring inclusion criteria. We sought data about the mother's age, parity, education, and employment, while for the child we confirmed sex and method of birth. The monitoring questions were assessed each month and consisted of infant general condition and breastfeeding status. To obtain body mass index data for mothers during pregnancy, data from the Mother and Child Health Handbook were used. Questionnaires were completed by the investigator after participants provided written informed consent.

Anthropometrical measurements

We measured the infant's body weight, length, and head circumference as anthropometric indicators. All measurements used metric scales and were performed at the Universitas Airlangga Hospital directly by investigator. The measurement was repeated once per month for three months. Measurement of body weight used an analogue infant scale with an accuracy of ± 0.1 kg. The infant scale was placed on a flat table that was not easy to sway. Before body weight was measured, the needle was set to zero. Babies were weighed naked without clothing and accessories. Body weight was recorded as the point where needle was most stable. Body length measurement was performed using an infantometer. The infants were first positioned lying on a flat bed. The infant's head was attached to the numerical barrier while the examiner's left hand pressed the knee straight and the right hand pressed the toe to the foot. Length was measured based on the pointed number on the outer edge of the gauge. Head circumference was measured with metered tape. The tape was looped over the forehead, between eyebrows, above the two ears, and passing the occiput. The metered tape was then pulled slightly tighter, and the value that crossed the zero point was read.

Breastmilk specimen collection

Breastmilk specimens were collected individually by each mother using a breast pump at random times. Full breastmilk expression, including foremilk and hindmilk, was used as a sample. To collect the sample, pumped breastmilk was first collected in a 50 mL sterile bottle. The minimum analyzable amount for each specimen was 3 mL, which was collected using sterile plastic tube. Collected breastmilk was then sent directly to the laboratory for analysis. We did not store any samples because they were all directly analyzed after collection. This procedure was repeated once a month for three months.

Breastmilk macronutrient analysis

Laboratory analysis was conducted at the Universitas Airlangga Hospital. Macronutrient analysis was performed using the Miris Human Milk Analysis instrument set (Miris, Sweden). Samples were first reheated to the optimal temperature and then homogenized with a 20 J/mL ultrasonic wave. Homogenized samples were analyzed under mid-infrared (mid-IR) transmission spectroscopy. Results are presented for lactose (g/100 mL), protein (g/100 mL), soluble fat (g/100 mL), and total calories (kcal/100 mL). The Pearson correlation coefficient was used to analyze the data using SPSS version 22.0. Results are presented in tables.

RESULTS

Subject characteristics

We enrolled 40 subjects for this study. A total of 10 participants were excluded, all due to non-exclusive breastfeeding. The remaining subjects finished all the observations and were included in the study. The mothers average age was 29.5 years old. Most of them were on their second parity (43%). The majority of the mothers were high school graduates (60%), followed by middle school graduates (17%). Most of the mothers (60%) were unemployed and taking a role as a housewife. With regards to the children, the number of male and female subjects was approximately the same (binomial non-parametric test, $p > 0.05$). Caesarean section occurrence was relatively high (47%) and served as the only assisted method of delivery in our study. Detailed information about subject characteristics is provided in Table 1, while the anthropometry summary is presented in Table 2.

Breastmilk macronutrient profile

We measured the breastmilk lactose, protein, fat, and total calories profile. We found that lactose was the most abundant breastmilk macronutrient (compared to protein and fat). The highest lactose concentration was detected in the third month of observations, with 4.17 ± 0.81 g/100 mL breastmilk. In our population, protein was the smallest component found in breastmilk. Its concentration peaked at the first month of observation at 1.89 ± 0.38 g/100 mL. We observed a slow but gradual decrease in the protein concentration each month. Fat was the second most abundant macronutrient; its concentration was the highest in the second month at 3.56 ± 0.96 g/100 mL, but dropped rapidly to 2.80 ± 1.16 g/100 mL (unlike the other macronutrients that did not exhibit such sharp temporal changes). Total breastmilk calories changed each month; it generally followed the same pattern as protein. The peak was 56.73 ± 9.77 kcal/100 mL in the first month, followed by a gradual decrease over the time. The complete breastmilk macronutrient profile is provided in Table 3.

Correlation between breastmilk macronutrients and infant anthropometry

Prior to performing the correlation tests, the Kolmogorov-Smirnov test was used to test the data distribution for all variables. All anthropometric measurements as well as breastmilk carbohydrates, protein, fat, and total calories were normally distributed ($p > 0.05$). Hence, the Pearson correlation coefficient was calculated for all variables. A detailed summary of the *p*-values, Pearson r correlation strength value, and the respective time of measurement is presented in Table 4.

We did not find any correlation between body weight and breastmilk macronutrients (carbohydrates, protein, or fat $p > 0.05$). We also did not find any correlation between breastmilk total calories and infant body weight ($p > 0.05$). These results were consistently found for all monthly observations.

Protein was related to infant growth, especially during the first month; protein was positively correlated with body length ($p < 0.05$) and head circumference ($p < 0.05$) for first month. However, there were no correlations between these measures and protein levels during the second and third months ($p > 0.05$).

There was also a positive correlation between breast milk total calories and infant head circumference for all three months of observations; ($p < 0.05$; Table 4).

DISCUSSION

During their first three months of life, infants grow faster than at older ages due to the larger body volume-to-surface ratio. Hence, young infants have an average higher average metabolic rate per kg body weight and require greater nutrient intake for body temperature regulation at this time.[12] This pattern of metabolism

TABLE 1. Participant characteristics.

Characteristic	Total (n = 30)
Mothers	
Age (years; mean ± SD)	29.5 ± 7.19
Parity	
First birth	7 (23%)
Second birth	13 (43%)
Third birth	5 (17%)
Fourth birth	3 (10%)
Fifth birth	2 (7%)
Highest Education	
Elementary school	3 (10%)
Middle school	5 (17%)
High school	18 (60%)
Vocational	3 (10%)
Bachelor's	1 (3%)
Mother's employment	
Housewife	18 (60%)
Employee	6 (20%)
Self-employed	6 (20%)
Infants	
Sex	
Male	17 (57%)
Female	13 (43%)
Mode of delivery	
Normal	16 (53%)
Caesarean section	14 (47%)
Birth weight (g; mean ± SD)	3123 ± 47.5
Birth length (cm; mean ± SD)	48.7 ± 0.50

TABLE 2. Infant anthropometric characteristics.

Components	Mean ± SD		
	1st month	2nd month	3rd month
Weight (kg)	3.46 ± 0.54 (normal[1])	4.51 ± 0.83 (normal[1])	5.60 ± 0.59 (normal[1])
Length (cm)	51.95 ± 2.40 (normal[1])	55.40 ± 2.11 (normal[1])	59.60 ± 2.14 (normal[1])
Head circumference (cm)	34.73 ± 1.16 (normal[1])	36.90 ± 1.11 (normal[1])	38.84 ± 1.10 (normal[1])

[1]Normal range is based on World Health Organization Z-score chart of body weight-for-age, length-for-age, and head circumference-for-age for children aged 0-2 years.

TABLE 3. Breastmilk macronutrients and total calories profile.

Components	Mean ± SD 1st month	2nd month	3rd month
Lactose (g/100 mL)	3.77 ± 0.72	3.72 ± 0.79	4.17 ± 0.81
Protein (g/100 mL)	1.89 ± 0.38	1.83 ± 0.43	1.72 ± 0.37
Fat (g/ 100 mL)	3.41 ± 0.97	3.56 ± 0.96	2.80 ± 1.16
Total calories (kcal/100 mL)	56.73 ± 9.77	55.50 ± 9.62	53.37 ± 10.72

TABLE 4. BCorrelations between breastmilk macronutrients and infant growth.

		Lactose		Protein		Fat		Total calories	
		r	*p*	r	*p*	r	*p*	r	*p*
Weight	1st month	0.051	0.79	-0.264	0.158	0.141	0.457	0.276	0.139
	2nd month	0.247	0.189	-0.037	0.847	0.152	0.423	0.269	0.151
	3rd month	0.488	0.189	-0.002	0.847	-0.023	0.423	-0.006	0.151
Length	1st month	0.291	0.118	0.501	0.005*	0.024	0.898	0.109	0.566
	2nd month	0.193	0.308	-0.044	0.817	0.101	0.597	0.283	0.129
	3rd month	0.577	0.308	-0.226	0.817	-0.177	0.597	-0.181	0.129
Head Circumference	1st month	0.222	0.238	0.468	0.009*	0.047	0.804	0.379	0.039*
	2nd month	0.278	0.136	-0.08	0.673	0.276	0.14	0.423	0.020*
	3rd month	0.445	0.136	-0.015	0.673	0.189	0.14	0.264	0.020*

Notes: r, Pearson's correlation coefficient; *statistically significant correlation ($p < 0.05$).

requires higher caloric input, and this need is met by macronutrients. Breastmilk is a natural source of such nutrition, and its content is critical for infant nutrition.

Weight and length are important anthropometric values in growing infants. We observed normal weight and length increases of exclusively breastfed infants during the study time interval. Our results differ from previous studies. One study showed 3.1% of infants were underweight in a cohort of exclusively breastfed infants at their third month of age[10], while another reported excessive overweightness in an exclusively breastfed cohort.[9,11] Our disparate results might be due to differences in subject characteristics, research methodology, and/or breastmilk quality. Hence, assessing breastmilk macronutrient levels is crucially important.

Head circumference has a relationship with brain volume, and it is often used to assess brain growth after a baby is born.[13,14] Evidence shows that total energy in breastmilk supports the energy requirements for brain growth,[15] which we will discuss later. However, the growth trajectory of the exclusively-breastfed infant brain is still poorly studied. Further studies are needed to assess how a specific breastmilk component could affect infant brain growth.

Compared to several previous studies in developed and developing countries, breastmilk in this study had lower lactose levels. Research in South Korea showed that breastmilk lactose levels in the third month averaged 7.1 ± 0.5 g/100 ml.[16] Similarly, research in the Philippines showed a higher mean lactose level, 7.3 ± 0.58 g/100 ml.[17] Lactose levels in breast milk are known to be higher in mothers who have more breast milk.[7] A study showed that formula feeding can increase the quantity of breast milk a breastfeeding mother produces.[18] This observation further reinforces the importance of maternal formula feeding during pregnancy.

In this study, the first month breastmilk fat content was lower compared to other reports. Of the analyzed macronutrients, our measured third-month fat level was the lowest when compared with other studies.[17,19] Fat content is the most variable macronutrient in breastmilk.[20] An Indonesian study of breastmilk fat levels reported an average of 5.94 ± 15.9 g/100 ml,[21] which is similar with studies conducted in South Korea (3.2 ± 1.0 g/100 ml)[16] and China (3.11 ± 1.13) g/100 ml).[22] These fat level differences are linked to variations in the maternal diet, age, type of analyzed samples (hindmilk or foremilk), breastfeeding time, and breastmilk storage conditions post-pumping.[16,22-25] Unfortunately, daily diet data and cohort data of maternal body mass index were not available in our study, and thus we could not confirm whether fat content variability in our samples was due to that factor.

In our study, breastmilk fat level was not directly correlated toward any child growth anthropometric measures. However, total calories were positively correlated to head circumference growth. Hence, we emphasize that both lactose and fat are important calorie sources used for brain development. There is limited evidence on how total caloric intake affects brain volume, which can be represented as head circumference. However, in the 1970s, a study showed that caloric intake is very important to achieve normal head circumference. The study showed that exposure to subnormal temperature (< 35°C) and failure to achieve a caloric intake of 120 cal/kg/day leads to abnormal head circumference growth in asymptomatic neonates. Recent findings showed that head circumference does not solely rely on total caloric intake. Rather, an adequate and balanced diet for optimal child brain development is recommended rather than pursuing a specific total caloric intake. The study showed that cumulative fat and caloric intake are positively associated with fractional anisotropy in the posterior limb of the internal capsule, a measure that relates to an increase in brain volume. The same study also showed that cumulative protein intake is positively associated with higher cognitive and motor scores.[26] Therefore, from these points of view and based on our findings, we conclude that breastmilk contains the necessary calories for adequate infant brain growth as well as the required macronutrients level needed to achieve daily caloric needs.

We observed a decrease in breastmilk protein levels as the infants aged. Protein levels contained in mature breastmilk are between 0.9-1.1 g/100 ml.[27] However, breastmilk protein levels are higher at the beginning of the lactation: they start at 1.4-1.6 g/100 ml, decrease to 0.8-1.0 g/100 ml when infants reaches 3-4 months old, and further decline to 0.7-0.8 g/100 ml when the baby reaches 6 months.[28] Interestingly, the breastmilk in this study had higher protein levels even during the third month of observation (1.72 ± 0.37) g/100 ml compared to that previous report. Again, maternal diet, age, type of analyzed samples (hindmilk or foremilk), breastfeeding time, and breastmilk storage conditions post-pumping might play role in our finding, since other macronutrients (especially fat) depend on those factors.[16,22-25]

Our findings on the correlation between breastmilk protein level and body weight suggest that protein is an important energy source in earlier months, but it is less crucial compared to fat and carbohydrates at older ages. There are several studies that support this view. One showed that weight gain is lower in infants who received breastmilk with less protein compared to higher protein. However, anthropometric measurements are higher in the group that received formula milk with higher protein, a measure that is associated with a higher-than-normal body mass index.[29] Our finding is also in line within the context of a study that stated protein intake in formula-fed infants exceeds the required amount after 1-2 months of age. Further, this excess is probably responsible for the higher adiposity in older infants. The same study also showed that the breastfed infants are leaner compared to other groups.[30] However, it must be noted that most of these previous studies compared protein levels of human breastmilk to formula milk. Therefore, careful deduction is still needed when concluding whether a difference in human breastmilk protein level alone might have the same effect.

Our study has some limitations that hinder us from drawing a more generalized conclusion. First, there is still no Indonesian standard regarding breastmilk macronutrient levels, a deficiency that makes our study prone to bias when compared to other studies due to differences in population characteristics. Second, although we used a total-sampling method to enroll participants, we were only able to register thirty participants (approximately one new participant per day). Further studies with longer sampling periods may be able to overcome this limitation. Third, our measurement method yielded no information on total breastmilk quality over 24 h, a deficit that made it difficult to calculate daily total calories available from the breastmilk. Fourth, perhaps the main drawback in our study was the unavailability of maternal diet data, which may affect breastmilk production and its quality during pregnancy and after childbirth. Finally, breastfeeding volume and frequency was defined "as the infant is satisfied with the breastfeeding" rather than a controlled bottled volume. Therefore, further studies with different designs are required to investigate about breastmilk and infant growth and overcome these limitations.

CONCLUSION

We conclude that breastmilk macronutrient levels correlate to infant growth in unique patterns. Breastmilk total calories were positively correlated with infant head circumference. Individual macronutrient levels yielded no significant correlations to infant body weight and length. Rather, the combined effect of the macronutrients manifested as total calories apparently affected growth. We also demonstrated that even lower concentrations of caloric sources (lactose and fat) compared to previous studies did not negatively affect infant growth. Therefore, we cannot fully support the idea that breastmilk causes underweight or overweight in infants less than 3 months old. Based on our findings, we support the idea of exclusive breastfeeding, since the evidence showed a positive relationship toward increased brain volume. Further studies that are able to overcome our limitations are needed to confirm our findings.

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