



# Breast Milk Macronutrients in Relation to Infants' Anthropometric Measures

Enas R. Abdelhamid<sup>1</sup>, Alyaa H. Kamhawy<sup>1\*</sup>, Alshaimaa A. Elkhatib<sup>1</sup>, Amr S. Megawer<sup>2</sup>, Amal I. El Shafie<sup>3</sup>, Yasmin G. El Gendy<sup>4</sup>, Dina E. A. Rabie<sup>4</sup>

<sup>1</sup>Department of Child Health, Medical Division, National Research Center, Dokki, Giza, Egypt; <sup>2</sup>Department of Pediatrics, Research Institute of Ophthalmology, Giza, Egypt; <sup>3</sup>Department of Health Radiation Research, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt; <sup>4</sup>Department of Pediatrics, Ain Shams University, Cairo, Egypt

## Abstract

**BACKGROUND:** Breast milk (BM) is the main nutritional source for newborns before they are capable to eat and consume other foods. BM has carbohydrates, lipids, complex proteins, and other biologically active components which have a direct effect on infant growth.

**AIM:** The aim of the study was to correlate anthropometric data of the infant to macronutrients in BM (fat, protein, and carbohydrates) and to find some modifiable issues affecting macronutrient contents of BM for the benefits of upcoming infants.

**METHODS:** One hundred breastfeeding mothers participated in the study, they were recruited from the outpatient clinic, El Demerdash Hospital, Ain Shams University, from September 2019, to December 2019. BM was expressed by an electric pump, macronutrient content was assessed. Anthropometric data of the babies and mothers were obtained, gestational age, parity, age of the women, and the route of birth were recorded.

**RESULTS:** For the macronutrients content of milk, a positive significant correlation was observed between BM fat, protein, and lactose. Infants' body mass index (BMI) was negatively related to the fat content of BM, while no relation was found between BMI and protein or lactose content of the milk. BM fat content was negatively correlated with gestational age and maternal age. Positive correlations were found between BMI and protein, lactose and infant age. Protein content was negatively correlated with parity. No impact of infant's sex on BM composition and as regards maternal diet, high protein consumption leading to increase BM protein content.

**CONCLUSIONS:** The current study confirms that BM macronutrient composition has a wide variability; this variability is associated with each macronutrient, respectively. To improve BM composition, one could aim for improving the nutritional balance in lactating women, especially for protein intake. More well-designed longitudinal studies about factors that influence human milk compositions are warranted.

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**\*Correspondence:** Alyaa H. Kamhawy, Researcher of Child Health, Department of Child Health, Medical Research Division, National Research Centre (NRC), 33 El-Bohouth St. (Former El- Tahrir St.), Dokki, Giza, Egypt.  
**E-mail:** alyaakamhawy@hotmail.com

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## Introduction

Breast milk (BM) is a rich complex nutrition source and is key to a baby's health, growth, and development [1].

Advantages of breastfeeding on the health and the infant wellbeing are well known, including prohibition of infections, optimal neurodevelopment, and may restrict the occurrence of allergy, diabetes, and obesity later in life [2], [3].

BM contains the main essential macro and micronutrients a human infant needs for early in life, the principal components of human milk (HM) are carbohydrates, proteins, lipids, vitamins, minerals, and other trace elements [4].

Carbohydrates are mostly lactose; several lactose-based oligosaccharides have been also known but as minor components. Fat portions have specific

triglycerides of oleic and palmitic acid, also lipids with trans bonds. The lipids have conjugated linoleic acid and vaccenic acid, which represent for up to 6% of the fat in HM [5]. Lipids in HM supply the infant with essential vitamins and energy, bioactive components, and polyunsaturated fatty acids. Recent studies showed that breastfeeding providing arachidonic acid and docosahexaenoic acid which improve cognitive development and reduce asthma risk at school-age children [6].

The principal proteins are IgA, lysozyme, alpha-lactalbumin, and lactoferrin (apo-lactoferrin) [7].

The World Health Organization encourages and maintains breastfeeding by their active recommendations that all infants must be exclusively breastfed for the first 6 months of life and that breastfeeding should be continued with the proper complementary foods for 2 years and beyond postpartum [8].

Nutritional components of HM derive from three sources: Some originate from the diet, some are created

from maternal stores, and some of the nutrients of milk originate by the synthesis in the lactocyte. Overall, the nutritional quality of HM is highly preserved, but care to the maternal diet is of great importance for the fatty acid composition and some vitamins of HM [9].

HM composition changes greatly between mothers and even within a single milk expression. This large variation in milk composition is supposed to be an adaptation to the infants' change in demands [10]. Moreover, it is influenced by numerous maternal, genetic, and environmental factors [4].

The colostrum and transitional milk in the early days of initiation of lactation vary greatly and are different in many ways from mature milk. Mature milk stays quite the same in composition with minor changes throughout the duration of lactation. The nutritional value is also different between preterm and term HM [11].

Breastfed infants increase largely in length, weight, and body mass index (BMI) during the first 2-3 months of newborn life and then reach a slower rate of growth over the next 12 months. They also have a larger fat accumulation in early infancy. Recent studies have found strong relations between BM composition (protein, total fat, adiponectin, leptin, HM oligosaccharides, and insulin) and velocity of infant growth. More studies, including studies of maternal factors affecting BM composition, are in need for more understanding how breastfeeding affects the present and final rate of growth and thereby short- and long-term health [12].

## Subjects and Methods

### Study design and target population

One hundred exclusively breastfeeding lactating women were recruited from September 2019 to December 2019 from outpatient clinic El Demerdash Hospital Ain Shams University. Written informed consent was taken in advance from the mother of each infant. The study had the ethical approval of the medical research committee at the National Research Center, having number 20\007.

### Infant anthropometric measurements

Infants were measured by a trained pediatric nurse, with weight and length. Weight was taken to the nearest 1 g using a Seca 757 electronic baby scale (Seca, Birmingham, UK). Supine length was calculated to the nearest 0.1 cm using a Seca 416 Infantometer (Seca, Birmingham, UK). BMI was then calculated (Tables 1 and 2) [13].

### Maternal data recording

Age of mothers, parity, and nutritional habits was recorded. Detailed dietetic history about food patterns, food intake, and regularity of meals was obtained.

### Milk collection and analysis

Women shared in the study donated their Hind BM samples through expression using Medela Symphony electric pump [14]. Milk samples were analyzed in the laboratory of the Dairy Sciences Department, Food Industry and Nutrition Research Division, National Research Center.

### Total fat determination

Fat percentage was detected according to British Standard Institution by Gerber method [15] by placing 10 ml  $H_2SO_4$  first into butyrometer then introduce of 10.75 ml of milk slowly, so the milk and  $H_2SO_4$  do not mix with each other. Finally we add 1 ml of Amyl alcohol. The three contents added vigorously then centrifuge for about 5 min, butyrometer then put in hot water bath for another 5 min, finally fat concentration was detected and measured [16].

Total protein determination: Protein concentration was assessed using the semi-micro Kjeldahl distillation method, according to Ling [17]. 5 gm of milk was put in into 100 ml flask. Then, it diluted to the mark with water, mix, and pipet 20 ml into a 300-ml Kjeldahl digestion flask. After adding of catalytic factors, sodium sulfate and nitrogen-free sulfuric acid were then added.

NaOH solution and distilled water were added, then the sample steam-distilled for approximately 10 min, adding of methyl red-methylene blue and a weak acid like a boric acid solution to receive ammonia [18].

Lactose determination: Lactose content was measured calorimetrically, according to Barnett and Abd El-Tawab [19]. As lactose is a disaccharide sugar which splits into glucose and galactose in the presence of lactase enzyme. Glucose reacts with a phenolic compound such as phenol-sulfuric acid through an enzymatic reaction, forming a pink colored complex. The resultant compound absorbance was read at 505 nm, of note that the color becomes darker as the lactose concentration increase.

**Table 1: Descriptive data of the studied infants and mothers**

Variable	Range	Mean $\pm$ SD
Infant age (by month)	6–14	9.40 $\pm$ 3.21
Gest. age (by week)	36–42	38.11 $\pm$ 1.21
Wt. (by kg)	6.5–11.5	9.15 $\pm$ 1.26
Length (by cm)	64–78	69.45 $\pm$ 4.39
Infants BMI	15.8–18.1	16.70 $\pm$ 1.20
Maternal age (by year)	24–30	27.46 $\pm$ 2.35

SD: Standard deviation, BMI: Body mass index.

Samples of milk were examined for total solids, true protein, fat, and lactose according to A.O.A.C. procedures. The ash content of milk was analyzed after a milk sample is heated in a muffle furnace at 550 °C for 8 h [20].

**Table 2: Food pattern and regularity of meals of the studied mothers**

Items	n	%
Breakfast regularly	43	73.0
Protein in breakfast regularly	55	55.0
Lunch regularly	92	92.0
Protein in lunch regularly	78	78.0
CHO in lunch regularly	86	86.0
Dinner regularly	64	64.0

### Statistical analysis

Analysis was performed using SPSS version 23.0 (SPSS Inc., Pennsylvania, USA). Mean  $\pm$  SD and percent were used for parameters' distribution calculations. Correlation was done using Person's correlation (Table 3).

**Table 3: Macronutrients in breast milk of the studied maternal sample**

Items	n=100
FAT (g/100 g milk)	
Mean $\pm$ SD	4.58 $\pm$ 0.94
Range	3–7.75
Protein (g/100 g milk)	
Mean $\pm$ SD	1.06 $\pm$ 0.28
Range	0.5–1.8
Lactose (g/100 g milk)	
Mean $\pm$ SD	6.52 $\pm$ 0.97
Range	4.7–9.4

SD: Standard deviation.

## Results

Negative significant correlation was detected between BM fat and infants BMI; furthermore, negative correlation was detected between breast milk fat and gestational age as shown in Figure 1.

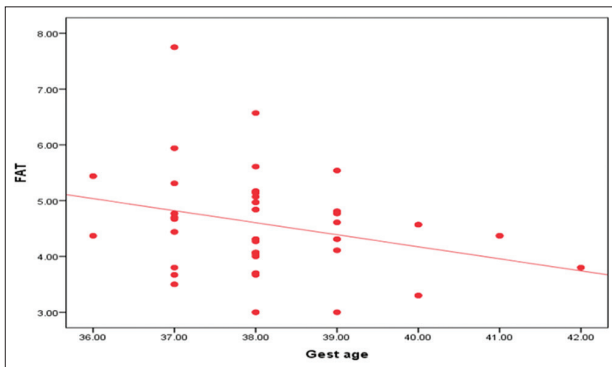


Figure 1: Relationship between breast milk fat and gestational age ( $r = -0.238$ )

While, a significant positive correlation was found between BM protein and infants' age. Furthermore, a highly positive significant correlation was found between BM lactose and infants' age (Table 4).

**Table 4: Relationship between breast milk macronutrients and different variables in the studied infants**

Variable	Breast milk fat		Breast milk protein		Breast milk lactose	
	r	p	r	p	r	p
Infant BMI	-0.210*	0.036	0.048	0.636	0.040	0.692
Infant age (by month)	0.087	0.390	0.235*	0.019	0.273**	0.006
Infant wt. (by kg)	-0.135	0.182	0.112	0.266	0.030	0.767
Infant length (by cm)	-0.008	0.936	0.099	0.327	0.001	0.991
Gest. age (by week)	-0.238*	0.017	0.121	0.230	-0.109	0.279

\*Significant difference ( $p \leq 0.05$ ); \*\*highly significant difference ( $p \leq 0.01$ ). BMI: Body mass index.

A significant negative correlation was detected between breast milk fat and maternal age. Furthermore, a significant negative correlation was detected between breast milk protein and parity (Table 5).

**Table 5: Relationship between breast milk macronutrients and different studied maternal variables**

Items	Breast milk fat		Breast milk protein		Breast milk lactose	
	r	p	R	p	r	p
Maternal variables						
Maternal age (by year)	-0.237*	0.017	0.134	0.185	0.040	0.692
Parity	0.020	0.846	-0.232*	0.020	-0.120	0.234
Mother wt. (by kg.)	0.032	0.752	-0.042	0.677	0.165	0.101
Mother height (by cm.)	0.229*	0.022	0.160	0.112	0.270**	0.007
Mother BMI	-0.105	0.299	-0.118	0.240	0.048	0.638

\*Significant difference ( $p \leq 0.05$ ); \*\*Highly significant difference ( $p \leq 0.01$ ). BMI: Body mass index.

Mean protein concentration in a group of mothers who got protein in lunch was higher than the other group who did not; the difference between both groups was highly significant (Table 6).

**Table 6: Breast milk protein content in relation to infant gender and maternal food patterns**

Variable	Protein Mean $\pm$ SD	Test value	p-value
Infant gender			
Male	1.09 $\pm$ 0.25	1.604	0.112
Female	0.99 $\pm$ 0.33		
Breakfast			
No	1.28 $\pm$ 0.28	5.430	0.000
Yes	0.97 $\pm$ 0.24		
Protein in breakfast			
No	1.15 $\pm$ 0.30	3.027	0.003
Yes	0.98 $\pm$ 0.24		
Lunch			
No	0.91 $\pm$ 0.26	-1.511	0.134
Yes	1.07 $\pm$ 0.28		
Protein in lunch			
No	0.92 $\pm$ 0.24	-2.676	0.009
Yes	1.09 $\pm$ 0.28		
CHO in lunch			
No	1.04 $\pm$ 0.28	-0.289	0.773
Yes	1.06 $\pm$ 0.28		
Dinner			
No	1.12 $\pm$ 0.25	1.628	0.107
Yes	1.02 $\pm$ 0.29		

\*Significant difference ( $p \leq 0.05$ ); \*\*highly significant difference ( $p \leq 0.01$ ). SD: Standard deviation.

## Discussion

Many studies have investigated how the composition of HM is in strong relation to infant growth. Recent observational researches have shown that BM concentrations of carbohydrate, fat, and protein are likely to have a strong influence on infant growth and body composition [21], [22].

The composition of HM is dynamic and complex, and many valuable considerations are important when discussing the strong association between infant growth and HM composition [9].

Studies showed that the amino acid composition of HM has an impact on growth, not only its concentration. Moreover, free amino acid concentration have an appetite regulating effect [23].

Many studies found that a higher intake of HM lactose can encourage the storage of glycogen and fat, and so promote growth [21].

It was suggested that fat percentage in HM may slow growth velocity, as it was associated with lower infancy weight and adiposity [23]

Analysis of the composition of mother's milk allows one to assess its nutritional value [14]. BM includes complex carbohydrates, proteins, lipids, and other biologically active components. The composition varies largely over a single feed as well as over the whole duration of lactation [24].

BM volume and composition are influenced by many factors such as delivery time, lactation stage [25], and prematurity which is shown to play a role also [26], recent studies reported that composition of BM could be tailored by each mother to meet the requirements of her infant [27].

Duration of breastfeeding is associated with reduced risk of developing obesity later in life [2], [28]. These observations suggest a dose-dependent effect of breastfeeding on the development of infant body composition, but the mechanisms of this effect are not fully understood [29], [30].

Our data revealed that fat content in BM was negatively correlated with infants' BMI, which fit that reported by Prentice *et al.* [23]. This could be explained by that low BM fat concentration makes the infant feel less satisfied and in need for increasing volumes of milk, hence more weight gain.

This work results showed that BM fat content was negatively correlated with gestational age, this was in concordance with a study of Fouad *et al.* [31]. Current results suggested that with a decrease in gestational age, the BM volume also decreases; consequently, the concentration of some nutrients such as fat appears to increase.

A negative correlation between BM fat content and maternal age was detected in the current study, which coincides with that of Fouad *et al.* [31]. The results of our study could be explained by exhaustion or depletion of body stores with a decrease of the amount of functional tissue in the breasts with the progression of age (disuse atrophy). In the contrary, studies of Heffner *et al.* [32] and of Lubetzky *et al.* [33] revealed that fat content did not affect by mothers' ages.

Current results documented a positive correlation between BM protein content and infant age, this finding could be explained by the adaptation of milk composition to the increased energy demand of the intensively growing child. Likewise, the study of Czosnykowska-Lukacka *et al.* [14] documented that the protein content of BM increased significantly with the duration of lactation. In the contrary, the study of Hascoët *et al.* [34] came with different results, as it reported that BM protein concentrations were negatively correlated with the duration of lactation from birth onwards.

This work showed a negative significant correlation between protein content of BM and parity, which is consistent with the publication of Bachour *et al.* [35], who showed that proteins decrease with increasing parity.

Suggested explanation is that the body reserve of proteins depletes with every successive pregnancy.

78% of our sample were taking protein on daily basis in their lunch meal, our results showed that this group had higher level of breast milk protein.

The current finding could be explained by direct transfer of maternal dietary proteins from plasma to BM which adds to the protein synthesized in the mammary secretory cell. In contrary to studies of Bauer and Gerss [36], and Aumeistere *et al.* [37], both revealed that concentration of HM protein is not affected by maternal diet.

A positive correlation between BM lactose content and infant age was detected in this study, the same as that of Hascoët *et al.* [34]. This could be explained by increasing milk volume with the age of the infant as a compensatory mechanism for increasing demand; this, in turn, is associated with an increase in lactose concentration.

BM carbohydrate content (lactose) was positively correlated with protein and fat content in our samples' BM. It fit the same reported by Hascoët *et al.* [34]. However, in contrary to Czosnykowska-Lukacka *et al.* [14], whose study showed a negative correlation between the main three macronutrients.

Our study reported no difference in BM composition in relation to infant's sex; likewise, van de Heijning *et al.* [38].

To the best of our knowledge, this is a point of conflict between many studies, the study of Hahn *et al.* [39] reported that BM was higher in energy for female infants in Korean and Kenyan mothers. In the contrary, the study of Powe *et al.* [40] found that mothers of male infants produced milk that had 25% greater energy content than mothers of female infants.

## Conclusion

Current study confirms that breast milk macronutrients composition has a wide variability, this variability is associated with each macronutrient respectively. To improve breast milk composition one could aim for improving the nutritional balance in lactating women, especially for protein intake. More well-designed longitudinal studies about factors that influence human milk compositions are warranted.

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