

## Assessment of the Breast Milk Triglyceride, Protein and Its Influencing Factors in Tehran, Iran

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### Abstract

**Introduction:** The composition of human milk is the biologic norm for infant nutrition. Human milk also contains many hundreds to thousands of distinct bioactive molecules that protect against infection and inflammation and contribute to immune maturation, organ development, and healthy microbial colonization.

**Methods:** 433 and 216 mothers were selected from Tehran southern and northern health care centers. The mother's diet was measured by a 24 hour diet history questionnaire for the past 3 days. All breast milk samples were frozen in plastic containers and stored immediately at  $-20^{\circ}\text{C}$  until analysis. The samples were centrifuged at 3000 g for 15 minutes and the superficial fat layer was separated. Breast milk protein was measured by the Lowry method. Breast milk Triglyceride was measured by the triglyceride colorimetric assay kit.

**Results:** The breast milk triglyceride and protein by mother's age by age, mother's occupation and mother's diet were mentioned in the tables.

**Discussion:** These findings suggest that milk composition is more sensitive to maternal factors such as age, weight, height, occupation, education and mother's diet. The assessment between mother's diet carbohydrate and protein between two groups showed a significant difference, statistically ( $P = 0.001$  and  $P = 0.000$ ) and also between the breast milk protein between two groups showed a significant difference, statistically ( $P = 0.028$ ). The assessment between the mother's diet fat in two groups was not significant ( $P = 0.069$ ) and also the assessment of the breast milk triglyceride between the two groups did not show a significant difference ( $P = 0.258$ ).

**Conclusion:** There was a significant difference between the mother's diet in the two groups and help us to change breast feeding malnourished mother's diet and convert it into a normal diet.

**Keywords:** Breast milk; Macronutrient; Protein; Triglyceride

### Introduction

The composition of human milk is the biologic norm for infant nutrition. Human milk also contains many hundreds to thousands of distinct bioactive molecules that protect against infection and inflammation and contribute to immune maturation, organ development, and healthy microbial colonization. The phenomenon of nutrition in early life having lifetime effects on growth, metabolism and health has been termed "nutritional programming" and has been defined as a long-term change in the structure or function of an organism resulting from a stimulus acting at a critical period of development in early life [1]. International agencies and various US health organizations uniformly recommend breastfeeding as the preferred method of infant feeding for the entire first year of life and thereafter as long as is beneficial to the mother and infant. The effect of nutritional status of mothers on the quality and quantity of their milk is a frequent topic of discussion. The composition of human milk can be affected by the diet consumed by the lactating woman. The influence of the maternal diet on milk composition varies in magnitude between nutrients; for some nutrients no effect at all has yet been documented [2,3]. It has been proposed that poor intrauterine growth not only contributes to increased morbidity and mortality during infancy, but also that it has the potential to compromise adult health and well-being. These recommendations are based on knowledge that term infants nursed by nutritionally adequate mothers are provided with sufficient energy and the proper profile of nutrients to support normal growth and development without any additional foods through the first 4 to 6 months of life. After 6 months, complimentary foods are needed to furnish nutrients likely to become limiting. Also human milk furnishes

an array of non-nutrient growth factors, immune factors, hormones and other bioactive components that can act as biological signals and confer protection against illness in infancy and later in life.

This phenomenon occurs when immaturity in tissues and organs involved in nutrient metabolism (i.e., the gastrointestinal tract, liver, and kidneys) limits the ability of an infant to respond to excesses or deficiencies in nutrient intakes. Human milk is species specific, and many of the nutrients it contains are secreted as bound components that can offer protection from digestion and facilitate absorption and utilization. The macronutrient composition of human milk varies within mothers and across lactation but is remarkably conserved across populations despite variations in maternal nutritional status. Lipids in comparison with the other nutrients of human milk have the greatest within and between sample variability. Studies on humans have shown the effect of maternal diet and anthropometric status on total milk fat content. The objectives of this study were to determine the possible relationship of breast milk fat content with maternal nutritional status.

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The mean macronutrient composition of mature, term milk is estimated to be approximately 0.9 to 1.2 g/dL for protein, 3.2 to 3.6 g/dL for fat, and 6.7 to 7.8 g/dL for lactose, 6.5-8% carbohydrates, 0.1-0.2% of electrolytes and the rest is water. Energy estimates range from 65 to 70 kcal/dL and are highly correlated with the fat content of human milk. A study examined the association between maternal characteristics and the composition of human milk macronutrient and found that after 4 months postpartum, the macronutrient concentrations of human milk are associated with one or more of the following factors: Maternal body weight for height, protein intake, parity, return of menstruation and nursing frequency [4].

Women who receive even a minimal basic education are generally more aware than those who are illiterate of the need to utilize available resources for the improvement of the health (particularly the nutritional status) of themselves and their families. In areas where cultural and religious beliefs seriously affect the mother's intake of food and or nutrients, every educational effort should be made to overcome these adverse practices. Special consideration must be given to ensure that intra-familial food distribution safeguards the mother's nutritional status.

Although maternal factors such as diet and body composition are plausible influences on this variation, most studies find weak or modest associations between maternal characteristics and the nutrient composition of the mother's milk. For instance, milk macronutrient composition appears to be largely independent of maternal diet and anthropometric measures of maternal body composition have inconsistent associations with milk composition. Milk protein is usually independent of body composition while the majority of studies report a modest association between body composition and milk fat, sugar, and total energy.

Associations between body fat and milk fat, if present, are usually positive, whereas milk sugar and maternal adiposity typically show an inverse relationship. Milk composition has also been shown to change over the course of lactation.

For the hypothesis, considering the significance of breast feeding, recognition of breast milk biochemical composition and their influencing factors is very important [5].

Information from such research will suggest strategies for nutrition intervention in areas of poor nutrition and provide dietary guidelines for lactating women [6-10].

The present study was performed to determine how and to what extent maternal nutritional status can be related to the breast milk amount and concentration of breast milk triglyceride and protein and produced by mothers of moderate to low socio-economic class and the changes in milk production.

The purpose of this article is to provide information for health care to guide the mothers.

The study protocol was approved by the Ethics Committee of Tehran University of Medical Science. All subjects were made aware of the content of the study and on agreeing to participate; a written informed consent document was obtained.

## Materials and Methods

### Subjects

The randomized sampling is easy and available. As shown in previous studies, the mean of protein in breast milk was  $3.7 \pm 1.3$  g /

100 ml. 650 samples are sufficient for predicting of mean of protein in breast milk, confident interval %95 and accuracy 0.1, as done by the following formula:

$$N = (\delta^2 Z_{\alpha/2}^2) / d^2$$

( $\delta = 1$ ,  $d = \%1.5 = \alpha$ , as the proportion of the population in the south to the north is about 2/1, so 433 and 216 mothers were selected from Tehran southern and northern health care centers, respectively.

### Milk collection and analysis

Socio-economic data (working status), demographic data and clinical data (health status) were obtained through an interview. All breast milk samples were frozen in plastic containers and stored immediately at  $-20^\circ\text{C}$  until analysis [11-15]. The samples were centrifuged at 3000 g for 15 minutes and the superficial fat layer was separated because high level of fat is an obstacle in accurate measurement [16,17]. Breast milk protein was measured by the Lowry method [13]. Breast milk triglyceride was measured by the triglyceride colorimetric assay kit. The assay is initiated with the enzymatic hydrolysis of the triglycerides by lipase to produce glycerol and free fatty acids. The glycerol released is subsequently measured by a coupled enzymatic reaction system with a colorimetric readout at 540 nm [14]. Information on mother's diet was collected by using a 24-hour recall method for 3 days (one week-end day included). Dietary intake of subjects was analyzed by Nutritionist III software programmer [18,19].

### Statistical analysis

Data for the human milk are presented as mean and standard deviation. One way ANOVA was applied to find differences between triglyceride and protein concentrations in two groups. The correlation between other factors (Mother's age, mother's occupation and mother's diet) and triglyceride and protein concentrations in human milk was shown as Pearson's correlation coefficient. All statistical analyses were done using STATISTICA 10.0.

## Results

Table 1 showed the breast milk triglyceride and protein in Tehran northern and southern health care centers. The breast milk triglyceride in northern was higher ( $499.68 \pm 251.07$  mg / 100 ml vs.  $466.77 \pm 205.33$  mg / 100 ml) than southern but the assessment of the breast milk triglyceride did not show a significant difference between the two groups ( $P = 0.258$ ).

The breast milk protein in southern was higher ( $0.99 \pm 0.45$  g / 100 ml vs.  $0.87 \pm 0.41$  g / 100 ml) than northern and there was a significant difference between the two groups, statistically ( $P = 0.028$ ). The breast milk protein in northern was less than RDA recommendations (0.9 to 1.2 g/dL).

Tables 2-4 showed the breast milk triglyceride and protein by age, occupation and mother's diet in Tehran northern and southern health care centers.

## Discussion

These findings suggest that milk composition is more sensitive to maternal factors diet in the first few months. Assessment of the breast milk biochemical compositions and its influencing factors such as age, weight, height, occupation, education and mother's diet between two Tehran northern and southern health care centers showed that the greatest part of mothers in northern (42.5%) and southern (38.2%) health care centers were 20-24 year old and there was not a significant

Breast milk	Northern (N = 216)	Southern (N = 433)	P-value
	Mean ± SD	Mean ± SD	
Triglyceride (mg/100 ml)	499.68 ± 251.07	466.77 ± 205.33	0.258
Protein (g/100 ml)	0.87 ± 0.41	0.99 ± 0.45	0.028

Significant (P < 0.05)

**Table1:** Breast milk triglyceride and protein in Tehran northern and southern health care centers.

Breast milk Age (year)	Northern (N = 216)		Southern (N = 433)	
	Triglyceride (mg/100 ml)	Protein (gr/100 ml)	Triglyceride (mg/100 ml)	Protein (gr/100 ml)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<20	538 ± 567.10	0.78 ± 0.48	509.06 ± 212.27	0.89 ± 0.32
21-25	483.75 ± 250.90	0.98 ± 0.43	477.07 ± 223.09	0.92 ± 0.37
26-30	482.63 ± 258.70	0.74 ± 0.36	457.52 ± 196.94	1.11 ± 0.61
31-35	620.94 ± 202.34	0.92 ± 0.42	419.47 ± 163.34	0.94 ± 0.32
>35	417.71 ± 208.87	0.79 ± 0.30	464.57 ± 227.60	1.39 ± 0.33
P-value	0.35	0.12	0.50	0.01

Significant (P < 0.05)

**Table2:** Breast milk triglyceride and protein by mothers age in Tehran northern and southern health care centers.

Breast milk Occupation		Triglyceride (mg/100 ml)	Protein (gr/100 ml)
		Mean ± SD	Mean ± SD
Groups: Northern (N=216)	Employed	517.20 ± 21	0.65 ± 0.40
	Housekeeper	498.74 ± 253.81	0.89 ± 0.41
	P-value	0.8	0.20
Southern (N = 433)	Employed	531.27 ± 198.55	1.14 ± 0.37
	Housekeeper	463.11 ± 205.60	0.98 ± 0.45
	P-value	0.29	0.26

Significant (P < 0.05)

**Table3:** Breast milk triglyceride and protein by mother's occupation in Tehran northern and southern health care centers.

Groups	Northern (N = 216)	Southern (N = 433)	P-value
	Mean ± SD	Mean ± SD	
Mothers' diet			
Carbohydrate (g/d)	329.48 ± 156.35	372.88 ± 182.10	0.001
Fat (g/d)	87.01 ± 50.92	94.10 ± 42.86	0.069
Protein (g/d)	73.13 ± 2.98	86.29 ± 49.10	0.000

Significant (P < 0.05).

**Table4:** The mothers diet in Tehran northern and southern health care centers.

difference between the two groups, statistically (P = 0.31). Assessment of mother's weight in the two groups showed that their weigh were 55-64 kg in northern (30.8%) and southern (29.7%) groups and there was not a significant difference between the two groups, statistically (P = 0.13). Assessment of mother's height in the two groups showed that they were 155-159 cm in northern groups (34.7%) and 160-164 cm in southern groups (34%) and there was not a significant difference between the two groups, statistically (P = 0.5). Assessment of education in the two groups showed that they have degree in northern (54.7%) and southern (44.6%) groups. The statistical tests did not show a significant difference between the two groups by education (P = 0.37) because they were illiterate of the need to utilize available resources for the improvement

of the health (particularly the nutritional status) of themselves and their families. In areas where cultural and religious beliefs seriously affect the mother's intake of food and or nutrients, every educational effort should be made to overcome these adverse practices. Assessment of mother's occupation in two northern and southern groups showed that most of them in northern (96.6%) and southern (94.1%) groups were housekeepers and there was not a significant difference between the two groups by occupation (P = 0.17). Assessment of the breast milk triglyceride and protein by mother's occupation in the two groups did not show a significant difference, statistically (P = 0.11). Assessment of mother's diet showed that the mother's diet carbohydrate, protein in southern was higher than northern and also the breast milk protein in southern was higher than northern. Mother's diet fat were higher in southern than northern but the breast milk triglyceride in northern was higher than southern while Mother's diet triglyceride was significantly inversely associated with breast milk triglyceride. Current maternal diet was not associated with milk composition.

The mother's diet carbohydrate in northern was less than southern (329.48 ± 156.35 g/d vs. 372.88 ± 182.10) and there was a significant difference between the two groups, statistically (P = 0.001). The assessment of the mother's diet protein between two groups showed a significant difference, statistically (P = 0.001 and P = 0.000) and also the assessment of the breast milk protein between two groups showed a significant difference, statistically (P = 0.028).

The assessment between the mother's diet fat in two groups was not significant (P = 0.069) and also the assessment of the breast milk triglyceride between the two groups did not show a significant difference (P = 0.258).

Therefore recognition of breast milk biochemical composition and their influencing factors can help us to change breast feeding malnourished mother's diet and convert it into a normal diet.

Human milk is a dynamic, multi-faceted fluid containing nutrients and bioactive factors needed for infant health and development. While many studies of human milk composition have been conducted, components of human milk are still being identified. Standardized, multi-population studies of the breast milk triglyceride and protein are sorely needed to create a rigorous, comprehensive reference inclusive of nutrients and bioactive factors.

In sum, we document considerable individual variation in human milk composition in these two group's mothers. Consistent with prior research, we find evidence that composition changes with the age of the mother and that this is likely partially reflecting changes in nursing frequency. Similar to past studies that report minimal evidence for associations between the milk nutritional variation and maternal diet or nutritional status as measured during lactation.

## Conclusion

There was a significant difference between the mean of mother's diet carbohydrate and mother's diet protein in the two groups and help us to change breast feeding malnourished mother's diet and convert it into a normal diet.

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