

# Comparison of long chain polyunsaturated fatty acid content in human milk in preterm and term deliveries and its correlation with mothers' diet

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**Background:** Human milk (HM) is the main food for infants, and phospholipids, especially long chain polyunsaturated fatty acids (LCPUFAs), play an essential role in the growth and brain development. This study was designed to evaluate the fatty acid composition in HM of mothers with preterm and full-term newborns and to determine the relationships of dietary intake of docosahexaenoic acid (DHA) and arachidonic acid (AA) of mothers and the content of these fatty acids in their milks. **Materials and Methods:** The AA and DHA of HM were determined by gas chromatography at the 3<sup>rd</sup> day after birth from mothers of 59 term and 58 preterm infants. Mothers were selected from those who delivered in Shahid Beheshti Hospital, a referral teaching hospital affiliated to Isfahan University of Medical Sciences, Isfahan, Iran. Dietary fat composition of mothers was examined by a food-frequency questionnaire. Total fat content, and DHA and AA levels of HM were compared in both groups. The correlation of dietary DHA and AA with DHA and AA of HM was determined in both groups. **Results:** We found that maternal age, body mass index (BMI), and self-reported food-frequency questionnaire did not differ in the two groups. The mean AA ( $0.19 \pm 0.10$  mg/ml and  $0.16 \pm 0.09$  mg/ml, respectively), DHA ( $0.10 \pm 0.06$  mg/ml and  $0.08 \pm 0.05$  mg/ml, respectively), and total fat content ( $2.58 \pm 2.16$  g/dl and  $2.06 \pm 1.22$  g/dl, respectively) of HM of mothers with preterm neonates were non-significantly higher than in mothers with term neonates. The percentage of DHA in the HM fat of preterm and term groups ( $0.45 \pm 0.16\%$  and  $0.45 \pm 0.18\%$ , respectively) and the percentage of AA ( $0.85 \pm 0.26\%$  and  $0.84 \pm 0.20\%$ , respectively) were comparable with worldwide standards. No correlations were documented between DHA and AA intake and DHA and AA content of HM in both groups. **Conclusion:** Although DHA and AA content of HM in preterm group was higher than in term group, this difference were not significant. In Isfahan, the percentage of DHA and AA was acceptable in the milk fat of mothers with term and preterm neonates.

**Keywords:** Human milk, polyunsaturated fatty acids, premature neonate

## INTRODUCTION

Human breast milk is the optimal nutrition for infants. Fats are the most important composition of breast milk, supplying the energy and, importantly, play a major role in the development of central nervous system.<sup>[1]</sup> The most significant and fundamental fatty acids are long chain polyunsaturated fatty acids (LCPUFAs) such as docosahexaenoic acid (DHA) and arachidonic acid (AA) which are available in breast milk. The infants have very limited ability to synthesize these fats from precursor. These fatty acids are added to the infant formulas in some developed countries, but most of the existing formulas do not have these essential fats.<sup>[2]</sup> The role of these lipids in the developing of brain and retina is clear. LCPUFAs are effective in the immune function, vision, cognitive and motor systems in the newborns. Studies have shown that visual acuity and cognitive development are better in breast feeding infants due to the presence of LCPUFAs in breast milk.<sup>[3]</sup> Generally, considerable amount of essential

fatty acids, mainly LCPUFAs, are transmitted from mother to fetus in the third trimester.<sup>[4]</sup> The brain tissue is rapidly synthesized during the last trimester of pregnancy and the early neonatal period. Cell differentiation and development of active synapses in the brain need specific requirements of DHA and AA. From the 26<sup>th</sup> week of gestation until birth, 80% of brain DHA forms in fetus. New researches have shown that DHA is important for normal development of brain glial cells.<sup>[5,6]</sup> The synthesis of these lipids is limited from the primary precursors such as linoleic acid (LA) and alpha-linoleic acid (ALA) in the fetus and neonate. The transmission of LCPUFAs from mother to fetus and newborn is through placenta during pregnancy and through breast milk after birth.<sup>[7,8]</sup> In premature birth, the transmission of LCPUFAs is interrupted from the placenta to the fetus, and infants should receive these fats exclusively from the breast milk. Studies have shown that the lipid composition of breast milk has considerable differences in different regions. It depends on diet, lifestyle of mothers, and the amount

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**Received:** 22-05-2012; **Revised:** 24-07-2012; **Accepted:** 03-12-2012

and nutritional supplements.<sup>[9,10]</sup> The lipids in breast milk for preterm infants are more important due to different aspects. However, the premature infants have lost the last trimester of pregnancy when most of the LCPUFAs are transferred to the fetus. On the other hand, premature infants after birth suffer from various problems that lead to frequent hospitalization. It is likely that many of these problems are due to the lack of adequate intake of LCPUFAs from the mother. The amount of fat in milk is different based on geographic conditions and dietary habits of the mothers, and consequently, the fat intake in premature infants is different. This could have a significant effect on the initial development of these infants.<sup>[10,11]</sup> Because of lack of new data on fatty acid composition of human milk in mothers with term and preterm neonates in Iran, especially in Isfahan, a central city in Iran, we performed a study to assess the level of milk lipids in breast milk fed by premature and term infants and to determine the relation between maternal diet and level of these fatty acids in breast milk. These data seem necessary for planning the neonatal health and nutritional recommendations at the community level.

## MATERIALS AND METHODS

From May 2010 until October 2010, 117 neonates and their mothers who delivered at Shahid Beheshti Hospital, a teaching hospital affiliated to Isfahan University of Medical Sciences, Iran, were enrolled in this study.

Based on the gestational age of neonates, we selected term and preterm neonates. The first group ( $n = 59$ ) included neonates who were delivered at term

(gestational age from 37 to 42 weeks) and the second group ( $n = 58$ ) consisted of preterm neonates, i.e., neonates with gestational age 28-36 weeks. Based on similar studies, 56 patients per group was found to be sufficient.<sup>[12]</sup>

The Ethics Committee in Isfahan University of Medical Sciences approved the study, and informed written consent was obtained from mothers before their accouchement.

Anthropometric data from mother and newborn pairs, as well as 20 ml of breast milk were collected on the third day after birth. Information on estimated gestational age, based on the date of last menstrual period, and characteristics of the birth and neonatal period were extracted from the medical records. The exclusion criteria included infants whose mothers were affected by certain diseases such as maternal hypertension, diabetes, advanced pregnancy, heart–kidney diseases, maternal medications (e.g., anticonvulsants or steroids), and patients with severe congenital anomalies. The infants with intrauterine growth retardation and

inappropriate weight for gestational age were excluded. The maternal age in both groups was from 18 to 30 years. Mothers who smoked and used nutritional supplements during pregnancy (such as fish oil) were excluded from the study. The questionnaires for the last 1 day of the maternal nutrition were also completed, and by using the existing software, the average of maternal daily DHA intake was estimated.

The breast milk samples were frozen at  $-70^{\circ}\text{C}$  until analysis. The fatty acid content was measured by using gas chromatography in the laboratory of the Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Shaheed Beheshti Medical University, Tehran, Iran.

## Statistical analysis

For statistical analyses, data were stored in a computer database and all analyses were performed using SPSS for windows version 11.5 (Chicago Inc., USA). Difference between means was assessed by independent samples *t*-test. Bivariate correlation between quantitative variables was tested by Spearman's coefficient. *P* values less than 0.05 were considered significant.

## RESULTS

Demographic and clinical characteristics of newborns and their mothers in both groups are shown in Tables 1 and 2. There was no significant difference in infants of both groups regarding the gender. They had significant differences in terms of birth weight, head circumference, height, and gestational age [Table 1]. On comparing the two groups of mothers, they had no significant difference in the following cases: Place of residence, education, occupation, weight before pregnancy, end pregnancy weight, height, body mass index (BMI) before pregnancy, and weight gain during pregnancy [Table 2]. The history of the mothers' daily diet was taken in both groups. The questionnaire was based on 1-day food consumption and daily consumption of the following: Energy, carbohydrate, protein, fat, saturated and unsaturated fats, linolenic acid, linoleic acid, oleic acid, DHA, cholesterol, and vitamin E. Mothers in both groups were not significantly different in terms of the consumption of food [Table 3]. Mother's milk was analyzed regarding AA, DHA, and total amount of fat. There was no significant difference in the mentioned cases in mothers of term and preterm infants [Table 4]. AA and DHA percentage content of total fat in the breast milk was calculated in the two groups. The percentage of AA in the total fat content of milk of mothers of premature infants was  $0.85 \pm 0.26\%$  (minimum of 0.17% and maximum of 1.71%) and it was  $0.84 \pm 0.20\%$  in mothers of term infants group (minimum of 0.55% and

**Table 1: Comparison of demographic characteristics of infants in both groups studied**

Variables	Term infants n=59		Preterm infants n=58		P values
	Number	Percentage	Number	Percentage	
Gender					
Male	34	57.6%	30	51.7%	0.52
Female	25	42.4%	28	48.3%	
Gestational age	37-42	38.96±1.13	28-36	33.34±1.13	<0.0001
Weight	2600-4000	3137.45±344.23	920-2700	1896.20±544.88	<0.0001
Height	45-55	50.63±2.37	31-52	43.86±4.42	<0.0001
Head circumference	32-37.5	34.75±1.16	21.5-34.5	30.39±2.92	<0.0001

**Table 2: Comparison of demographic characteristics of mothers in both groups studied**

Variables	Term infants n=59		Preterm infants n=58		P values
	Number	Percentage	Number	Percentage	
Residence					
Urban	52	88.1	52	89.6	0.58
Rural	7	81.9	6	10.4	
Education					
Illiterate	2	3.4			0.07
Reading and writing	11	19	12	20.7	
School	11	19	9	15.5	
Diploma	26	44.8	26	44.8	
Associate degree	7	12.1	2	3.4	
BA	1	1.7	8	13.8	
MA			1	1.7	
Occupation					
Worker			1	1.7	0.49
Employee	3	5.1	4	6.9	
Dr.			1	1.7	
Free			1	1.7	
Housekeeper	56	94.9	52	87.9	
Weight before pregnancy	40-95		40-98		0.94
	63.64±13.04		63.81±11.92		
Weight at birth	49-108		45-101		0.55
	76.03±14.16		74.56±12.37		
Height	145-178		148-177		0.11
	160.59±5.86		162.34±6.10		
BMI before pregnancy	15.99-36.05		16.65-34.72		0.62
	24.68±4.95		24.25±4.61		
BMI at birth	20.45-42.22		18.73-39.45		0.21
	29.47±5.29		18.73-39.45		
Weight gain during pregnancy	2-22		4-28		0.67
	11.69±3.79		11.37±4.32		

BMI=Body mass index

maximum of 1.43%). The percentage of DHA in the total fat content of breast milk in preterm infants group was  $0.45 \pm 0.16\%$  (minimum of 0.12% and maximum of 0.88%) and in the term infants group was  $0.45 \pm 0.18\%$  (minimum of 0.13% and maximum of 1.07%).

The mean maternal dietary intake of DHA did not correlate with DHA of the breast milk ( $r = 0.044$  and  $P = 0.64$ ) when data of all newborn infants and mothers were considered together. When this relationship was also evaluated in separate groups, there was no relationship between the

maternal DHA intake in premature infants group and their milk DHA ( $r = -0.031$  and  $P = 0.81$ ) and in the term infants group whose mothers received DHA and their milk DHA ( $r = -0.022$  and  $P = 0.87$ ).

## DISCUSSION

In this study, the levels of long chain polyunsaturated lipids in the breast milk of mothers of term infants (gestational age from 37 to 42 weeks) and preterm infants (gestational age from 28 to 36 weeks) were evaluated. It was observed that

**Table 3: Comparison of the mean of daily nutrients in maternal food consumption based on the questionnaires filled by mothers in both groups studied**

Variables	Term infants	Preterm infants	P values
	n=59	n=58	
Energy (kcal)	24.63-3397.00 1944.94±262.18	818.30-3243.00 167.66±610.28	0.84
Carbohydrate	120.50-53.50 288.19±85.93	150.90-2266 342.14±275.03	0.15
Protein	22.09-135.60 86.24±28.33	14.51-155.70 79.16±35.35	0.23
Fat	15.92-109.20 55.69±24.30	11.86-102.40 49.93±21.71	0.17
Saturated fat (SFA)	6.21-48.26 20.95±0.02	4.51-41.67 19.34±9.35	0.37
Unsaturated fats (poly) (PUFA)	0.73-29.95 8.47±4.93	0.89-23.28 7.85±4.99	0.49
Unsaturated fats (mono) (MUFA)	3.94-1815.00 50.33±233.93	0.7-115.74 17.99±15.72	0.29
Linolenic acid (ALA) (EFA, W3)	0.00-0.89 0.20±0.18	0.00-7.00 0.38±0.99	0.16
EPA (LCPUFA, W3)	0.00-0.32 0.04±0.09	0.00-0.39 0.02±0.08	0.25
DHA (LCPUFA, W3)	0.00-0.87 0.12±0.25	0.00-1.01 0.075±0.41	0.23
Linoleic acid (LA) (EFA, W6)	0.51-40.60 7.30±6.18	1.67-20.30 6.12±4.38	0.23
Oleic acid (oleic) (MUFA)	0.37-47.33 14.63±9.50	1.51-2061.00 49.19±269.21	0.32
Cholesterol	32.25-835.90 254.75±146.02	27.56-468.70 212.93±102.10	0.07
Vitamin E	0.09-10.17 3.24±1.66	0.92-27.05 3.71±3.33	0.34

LCPUFA=Long chain polyunsaturated fatty acids; ALA=Alpha-linolenic acid

**Table 4: Comparison of the mean fat content of breast milk in both groups**

Variables	Term infants	Preterm infants	P values
	n=59	n=58	
Arachidonic acid (LCPUFA W6) mg/ml	0.04-0.49 0.16±0.09	0.02-0.73 0.19±0.10	0.15
DHA (LCPUFA W3) mg/ml	0.02-0.35 0.08±0.05	0.02-0.33 0.10±0.06	0.07
Total amount of fat g/dl	0.28-6.03 2.06±1.22	0.33-14.36 2.58±12.16	0.11

although the mean of AA, DHA, and total fat in the milk of mothers of premature infants was more than in the milk of mothers of term infants, this difference was not significant. Our study also showed that DHA and AA levels in the breast milk of mothers of both term and premature infants were at the levels of standards reported elsewhere around the world. A meta-analysis (including 106 studies on maternal milk lipids) has shown that the mean of DHA in breast milk was  $0.32 \pm 0.22\%$  and the mean of AA in breast milk was  $0.47 \pm 0.13\%$ .<sup>[13]</sup> In our study, the mean DHA in the breast

milk of mothers of preterm infants was  $0.10 \pm 0.06\%$  and it was  $0.08 \pm 0.05\%$  in the breast milk of mothers of term infants. The mean AA was  $0.19 \pm 0.10\%$  in the milk of mothers of premature infants and  $0.16 \pm 0.09\%$  in the milk of mothers of term infants. However, there are no reports from different parts of Iran regarding the lipids in breast milk; further researches in other areas seem to be necessary. A study conducted in Buenos Aires in Argentina in 2009 compared the fat composition of milk of mothers with premature infants (28-36 weeks) and term infants (37-42 weeks). It was shown that unsaturated and saturated lipids in the milk of mothers of premature infants were more than in the mothers of term infants.<sup>[14]</sup> This study also showed that the gestational age affects milk fat composition. The levels of saturated lipids as the energy source and unsaturated lipids, which are necessary for brain development (preterm infants have a greater need for them), were higher in mothers of premature infants. In another study in Hungary, two groups of premature and term infants and their mothers were considered. Age, BMI, and dietary habits of mothers in the two groups did not differ. LA and ALA in breastfed term and premature infants did not differ, even though AA and DHA in the breast milk were higher in preterm infants group. The study also showed that because of its unsaturated fat content, the breast milk of mothers with preterm infants was more useful than the milk of other mothers for premature infants.<sup>[15]</sup> Our study also showed that although AA and DHA levels in the milk of mothers of term and preterm infants had no significant difference, the level of fat in the milk of mothers with preterm infants was higher. Probably, by conducting more studies (taking more samples), this difference could be found significant. Our results showed that daily intake of saturated and unsaturated lipids by mothers of term and preterm infants did not differ and had no role. Moreover, the levels of long chain unsaturated fatty acids were not different in the breast milk of mothers with preterm infants and those with term infants and it was not associated with the daily fat intake of mothers.

In a study in 2008 in four regions of Thailand, it was also shown that DHA in breast milk varies in different regions of Thailand. The Thailand study, as well as our study, showed that there was no relation between daily intake of DHA and DHA levels in breast milk. The probable cause of differences in maternal milk DHA could be the difference in the mother's diet or genetic factors.<sup>[16]</sup> In another study comparing the DHA content of breast milk in nine countries (Australia, Canada, Chile, China, Japan, Mexico, Philippines, England, and the United States), it was shown that the AA was similar in all countries, but DHA was significantly different in these countries. The highest was in Japan and the lowest in Canada and the United States.<sup>[17]</sup> In a study conducted in Berlin, Germany, it was shown that by the administration of 200 mg daily DHA to mothers who used less fish in their diet, the percentage of DHA in the red



blood cells of infants and their mothers was significantly increased.<sup>[18]</sup> Considering the above-mentioned studies, it was found that the amount of milk lipids in different areas is different and depends on diet and genetic factors. However, the administration of dietary supplements changes the amount of fat in breast milk and infants.

## CONCLUSION

The important result of this study is that the amount of useful fats of breast milk were acceptable in our region. It seems that further studies are needed for educating mothers to take supplements or foods with high unsaturated lipids. This could increase the amount of fat in the breast milk and could be an essential step in improving the infant health, and also for better development of neurological system and health promotion.

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**How to cite this article:** Iranpour R, Kelishadi R, Babaie S, Khosravi-Darani K, Farajian S. Comparison of long chain polyunsaturated fatty acid content in human milk in preterm and term deliveries and its correlation with mothers' diet. *J Res Med Sci* 2013;18:1-5.

**Source of Support:** This study was conducted as a specialty thesis funded by the Vice-chancellery for Research and Technology, Isfahan University of Medical Sciences, Isfahan, Iran, **Conflict of Interest:** None declared.