ORIGINAL ARTICLE

A study on lipid content and fatty acid of breast milk and its association with mother's diet composition

Roya Kelishadi, Bagher Hadi, Ramin Iranpour, Kianoush Khosravi-Darani[1], Parisa Mirmoghtadaee, Sanam Farajian[2], Parinaz Poursafa[3]

Department of Pediatrics, Child Growth and Development Research Center and Faculty of Medicine, [2]Departments of Clinical Nutrition, School of Nutrition and Food Science, and Food Security Research Center, [3]Environmental Health, Environment Research Center, Isfahan University of Medical Sciences, Isfahan, [1]Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Shaheed Beheshti Medical University, Tehran, Iran

Introduction: The aim of our study was to determine the content of fat and fatty acid composition of breast milk , and its association with the mother's diet. **Materials and Methods:** This cross-sectional study was conducted among pregnant mothers who came to health care centers for last prenatal care in Isfahan, Iran. Eight to 72 hours after delivery, 2 to 5 ml of colostrum was collected by hand into tubes. They were kept in an ice box and sent within half an hour to the collaborating health centre for freezing at -20°C until analysis, which was performed at the laboratory of NNFTRI in Tehran. The milk samples were homogenized by Vortex (Heidolph Vortex Shaker REAX 1. 220 V. 30 W Germany) at 2400 rpm for 30 sec. **Results:** The data of 86 out of 91 samples were complete. The mean maternal age and gestational age was 28.37 ± 5.55 years old and 38.7 ± 1.2 weeks, respectively. The content of fat was 2.17 ± 1.22 g/100 ml breast milk. Arachidonic acid (AA, 20:4n-6) and docosohexaanoic acid DHA (22:6n-3) made 0.8 ± 0.4% and 0.3 ± 0.2% of total fatty acids. Although the AA/DHA ratio in our study is suitable, but the content of DHA is nearly low. **Conclusion:** Dietary habits of women in reproductive age group should be improved, with special emphasis on the fatty acid content of breast milk. This may have long-term impact on health promotion and disease prevention.

Keywords: Breast milk, fatty acid, Iran, neonate, pregnancy

INTRODUCTION

Exclusive breast feeding is the best choice for full term infant nutrition. The role of fat and fatty acids (FA) in human breast milk as main source of energy and essential lipid soluble vitamins and in optimum infant development was shown in many studies.[1-3] Poly unsaturated fatty acids (PUFA) such as Linoleic acid (LA) (18: 2n-6) and α-Linolenic acid (ALA) (18:3n-3) are precursors of long chain poly unsaturated fatty acids (LCPUFA) such as arachidonic acid (AA; 20:4n-6) and Docosohexaanoic acid (DHA; 22:6n-3). They are necessary for growing brain and the retina during perinatal development.[2,4]

It is well established that current and long-term mother's diet, individual and diurnal variations in fatty acid synthesis, genetic factors, stage of lactation influences on fatty acid composition of breast milk.[1,2,4] And due to various dietary habits and life styles worldwide, fatty acid composition of breast milk is different not only within countries but also within regions of a country.,[1,5-9] In a study among German women, the percentage of AA and DHA was 0.7% and 0.46% of total fatty acid in mature breast milk, respectively.[10] In another study, DHA increased with consumption fatty sea fish or by supplementation with fish oil.

In Iran, little is known of the FA composition in the milk of Iranian women and its relations to their diet. A study in the west of Iran showed the fat content and the total PUFA in milk samples of 52 women were 3.8 ± 1.5% and 16.5%, respectively.[11]

The aim of our study was to determine the content of fat and AA and DHA of breast milk as two major LC-PUFA connected with favorable outcomes in human infants and to associate these results to the mother's diet such as carbohydrate, protein, calorie intake and lipid composition of colostrum as first food of newborns.

Access this article online	
Quick Response Code:	Website: www.journals.mui.ac.ir/jrms
	DOI: ***

Address for correspondence: Prof. Roya Kelishadi, Department of Pediatrics, Child Growth and Development Research Center and Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: kelishadi@med.mui.ac.ir

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

MATERIALS AND METHODS

This cross-sectional study was done among pregnant mothers who came to health care centers for last prenatal care in Isfahan, Iran. They were selected by convenient sampling. Pregnant women with gestational age between 37 to 42 weeks, without chronic disease and drug use (only routine supplements use of iron and folic acid) included the study. Neonates with congenital anomaly, intrauterine growth retard and small for gestational age were excluded from the study.

The Research and Ethics Committee of Faculty of Medicine, Isfahan University of Medical Sciences approved the study. Written consent was obtained from all mothers. They completed one-day food record questionnaire to determine their current food intake and energy, protein, carbohydrates, fat and fatty acids using nutrition software.

After giving a clean plastic disposable storage box, the researcher explained mothers to bring 20cc of their breast milk in it to neonatal screening clinic after delivery. In Iran all neonates was screened for congenital hypothyroidism in 1-5 days after delivery. Then the samples were delivered to Pharmacy School of Isfahan University of Medical Science.

Breast milk sampling and fatty acid analysis

When the mothers were in the post-partum ward, 8 to 72 hours after delivery, 2 to 5 ml of colostrum was collected by hand into tubes. They were kept in an ice box and sent within half an hour to the collaborating health centre for freezing at -20°C until analysis, which was performed at the laboratory of NNFTRI in Tehran. The milk samples were homogenized by Vortex (Heidolph Vortex Shaker REAX 1. 220 V. 30 W Germany) at 2400 rpm for 30 sec.

Direct FAME synthesis

About 1 ml of milk were placed into a 16 × 125 mm screw-cap Pyrex culture tube to which 1.0 mL of the C19:0 internal standard (1 mg of C19:0/mL of MeOH), 0.7 mL of 10 N KOH in water, and 5.3 mL of MeOH were added. The tube was incubated in a 55°C water bath for 1.5 h with vigorous hand shaking for 5 s every 20 min to properly permeate, dissolve, and hydrolyze the sample. After cooling below room temperature in a cold tap water bath, 0.58 mL of 24 N H_2SO_4 in water was added. The tube was mixed by inversion and with precipitated K_2SO_4 present was incubated again in a 55°C water bath for 1.5 h with hand-shaking for 5 s every 20 min. After FAME synthesis, the tube was cooled in a cold tap water bath, 2 milliliters of hexane was added, and the tube was vortex-mixed for 2 min on the vortex. The tube was centrifuged (Rotina 35 R, Hettich, Tuttingen, Germany) for 2 min at 5000 rpm at 4°C and Na_2SO_4 (1 g) was added to the upper phase contained fatty acid methyl esters (FAME) and it was removed to clean test tubes.

Gas chromatography (GC)

The vial was capped and placed at −20°C until GC analysis; 1 µl of the esterified sample was injected into the gas chromatography (GC-CP3800, Varian, USA) equipped with a flame ionization detector and a fused silica capillary column 50 m × 0.25 mm (I.D. CP-SIL 88 for FAME) supplied with 0.2µm stationary phase (Varian Chrompack, USA-Netherlands). Oven temperature was maintained at 100°C for 2 min, then increased to 10°C/min to 175°C and held there for 15 minutes followed increase at 5°C/min to 205°C and followed increase 3°C/min to 220°C and kept there for 24.5 min (total time: 60 min). The injector and the detector temperatures were set at 260°C and 270°C, respectively. Helium was the carrier gas with a split ratio of 30: 1 and a column flow of 0.5 ml/min.

Fatty acids were identified by comparing the retention times of FAME with a standard FAME mixture (SUPELCO 37 Comp. FAME mix, (595, North Harrison Road, Bellefonte, Pennsylvania (PA) 16823-0048, USA). All samples were analysed in duplicate. The results were expressed as weight/weight area percent. The values were given as means (SD).

Statistical analysis

Results were evaluated with SPSS software version 17.0. Descriptive analysis was done to determine the frequency, the mean ± SD of some variables. Pearson correlation test was used to test the correlations between total fatty acids and mothers diet composition.

RESULTS

The data of 86 out of 91 samples were complete. The mean of age and gestational age was 28.37 ± 5.55 years old and 38.7 ± 1.2 weeks, respectively. Mother's diet composition is presented in Table 1.

Table 1: Diet composition of pregnant mothers in food frequency questionnaire

Diet composition	Minimum	Maximum	Mean	Std. deviation
Energy (Kcal/Day)	24.63	4310.00	1947.46	647.88
Fat (mg/day)	15.03	166.70	56.91	25.81
Saturated fatty acids(mg/day)	6.21	91.37	22.13	11.98
PUFA (mg/day)	0.73	29.95	8.85	5.35
MUFA (mg/day)	3.72	1815.00	39.48	189.53
EPA (mg/day)	0.00	3.84	0.075	0.40
DHA (mg/day)	0.00	0.87	0.09	0.21
Oleic acid (mg/day)	0.37	47.33	13.96	9.094
Linoleic acid (mg/day)	0.51	40.60	7.58	6.06
Cholesterol (mg/day)	14.24	835.90	251.80	148.39
Carbohydrate (mg/day)	120.50	2266.00	315.32	226.42
Protein (mg/day)	22.09	201.10	84.86	30.68

PUFA = Poly unsaturated fatty acids; MUFA = Monounsaturated fatty acids; EPA = Eicosapentaenoic acid; DHA = Docosohexaanoic acid

The content of fat was 2.17 ± 1.22 g/100 ml breast milk. AA (20:4n-6) and DHA (22:6n-3) made 0.8 ± 0.4% and 0.3 ± 0.2% of total fatty acids.

The correlations between fat content of breast milk and some components of mother's diet were presented in Table 2.

DISCUSSION

Our results suggested that the content of fat in 100 cc of breast milk is lower than many studies which reported its average between 3.8-3.9 g/100 ml.[12] We did not find any study in Iran that evaluated the fat content of breast milk in first 5 days of lactation. In a study in the west of Iran, the fat content in the milk of 52 healthy women was reported 3.8 ± 1.5% in the 45th to 155th day of their lactation.[11] Another study in the north west of Iran in Tabriz among mothers with exclusively breastfed infants aged 90-120 days showed the mean fat content of breast milk samples was 3.52 ± 1.41 g/ dl). It was well established that total fat content of breast milk increased with the duration of lactation.[2,4,13]

In a study in Germany, the content of 4n-6 and 6n-3 in 38 term mothers was reported 0.72% and 0.45% of total fatty acids in 5 days of their lactation.[10]

In our study, the milk AA content was lower in comparison with Western part of Iran. In other studies it was shown that the percentage of LC-PUFA of both n-6 and n-3 derivatives decrease during the first month after child birth.[2,4] In addition, we found association between AA content of breast milk and mother's diet composition. Many studies showed that the milk AA content may be less sensitive to maternal dietary intakes and it is synthesized from essential fatty acids in mammary glands.[1,2,4]

The content of DHA in our study is low in comparison to other studies. It was reported to be low as 0.2 wt.% in Italy to as high as 1.1 wt.% in ST.Luica in other investigations.[8,9] No correlation was found between breast milk DHA and DHA of mother's diet did not confirm the influences of the diet of lactating women. Many studies showed that consumption of fatty sea fish or supplementation with fish oil increased the DHA content of breast milk.[2,4,9,14]

These differences might be due to using different methods in extraction of lipids and fatty acids of breast milk and different programs for mother's diet analysis. In addition, genetic and geographical differences, which documented as important factors on fat content of breast milk, should be considered.[2,4]

WHO recommended AA/DHA ratio should be 2:1 in colostrums. In well developed country, this ratio varies from 5:1 to 15:1.[4] It was 2.6:1 in our study higher than WHO recommendation.

Although the AA/DHA ratio in our study is suitable, but the content of DHA is nearly low. Because of its important role in infant brain development interventions to improve dietary habits in before and during pregnancy is needed. It was shown that in regions where fish consumption is high, the DHA content of breast milk is higher.[9,15]

In this study, we found no association between measured breast milk fat content and daily energy intake, carbohydrate, protein, linoleic acid, linolenic acid, saturated fatty acid, PUFA, monounsaturated fatty acid, and cholesterol of mother's diet. For mature milk, the correlation of carbohydrate of mother's diet and fat content was shown in Tabriz.[16] But there are many researches which showed no correlation between breast fat content and energy intake, carbohydrate, protein of mothers' diet in mature milk and colostrums.[17-19] Breast feeding should be promoted for prevention of chronic diseases,[20] and consumption of foods rich in fatty acids should be encouraged for women in reproductive age group.

Table 2: Correlations between breast milk fatty acids and mother's diet composition

Breast Milk (mg/d)		Mothers diet composition											
		Fat	Energy (mg/d)	Protein (mg/d)	Carbohydrate (mg/d)	Cholesterol (mg/d)	LA (mg/d)	ALA (mg/d)	MUFA (mg/d)	PUFA (mg/d)	SFA (mg/d)	EPA (mg/d)	DHA
DHA	Pearson correlation	0.08	0.11	0.140	−0.05	−0.002	0.15	−0.07	0.08	0.08	0.07	0.06	.09
	P value	0.47	0.30	0.211	0.64	0.98	0.18	0.520	0.48	0.46	0.51	0.53	.39
AA	Pearson correlation	−0.0.04	-0.01	0.027	−0.09	−0.08	0.12	−0.03	0.04	0.07	−0.03	0.13	.22*
	P value	0.67	0.90	0.813	0.4	0.42	0.26	0.761	0.69	0.53	0.75	0.22	.04
Fat content (%)	Pearson correlation	−0.02	0.009	0.096	−0.10	−0.06	0.16	−0.01	−0.01	0.11	−0.01	0.286*	.28**
	P value	0.85	0.93	0.39	0.37	0.58	0.13	0.88	0.89	0.30	0.92	0.01	.009

*Correlation is significant at 0.05 level; **: Correlation is significant at 0.01 level LA = Linoleic Acid; ALA = α-Linolenic Acid; SFA = Saturated Fatty acid; MUFA = Monounsaturates Fatty Acid; PUFA = Poly unsaturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; EPA = Eicosapentaenoic acid; AA = Arachidonic acid; DHA = Docosohexaanoic acid

Using the one-day questionnaire is one of the limitations of the study. This questionnaire determined the current dietary of pregnant mothers. It was well established that not only current diet, but also long term dietary habit influences the human breast milk fatty acid composition especially DHA.

CONCLUSION

Dietary habits of women in reproductive age group should be improved, with special emphasis on the fatty acid content of breast milk. Consumption of oily fish, nuts, seeds, whole grains, egg, dairy, and dark green, leafy vegetables may be helpful in this regard. This may have long-term impact on health promotion and disease prevention.

REFERENCES

1. Samur G, Topcu A, Turan S. Trans fatty acids and fatty acid composition of mature breast milk in turkish women and their association with maternal diet's. Lipids 2009;44:405-13.
2. Koletzko B, Rodriguez-Palmero M, Demmelmair H, Fidler N, Jensen R, Sauerwald T. Physiological aspects of human milk lipids. Early Hum Dev 2001;65 Suppl:S3-S18.
3. Birch EE, Castan˜eda YS, Wheaton DH, Birch DG, Uauy RD, Hoffman DR. Visual maturation of term infants fed long-chain polyunsaturated fatty acid–supplemented or control formula for 12 mo. Am J Clin Nutr 2005;81:871-9.
4. Fidler N, Koletzko B. The fatty acid composition of human colostrums, Eur J Nutr 2000;39:31-7.
5. Peng Y, Zhou T, Wang O, Liu P, Zhang T, Zetterstöm R, *et al*. Fatty acid composition of diet, cord blood and breast milk in Chinese mothers with different dietary habits. Prostaglandins Leukot Essent Fatty Acids 2009;81:325-30.
6. Xiang M, Harbige LS, Zettersreom R. Long-chain polyunsaturated fatty acids in Chinese and Swedish. mothers: Diet, breast milk and infant growth. Acta Paediatr 2005;94:1543-9.
7. Fidler N, Salobir K, Stibilj V. Polyunsaturated fatty acid composition of human colostrum lipids in slovenia: Regional differences. Lipids Food Technol Biotechnol 2000;2:149-53.
8. Serra G, Marletta A, Bonacci W, Campone F, Bertini I, Lantieri PB, *et al*. Fatty acid composition of human milk in Italy. Biol Neonate 1997;72:1-8.
9. Boersma ER, Offringa PJ, Muskiet FA, Chase WM. Vitamin E, lipid fractions, and fatty acid composition of colostrum, transitional milk, and mature milk: An international comparative study. Am J Clin Nutr 1991;53:1197-204.
10. Genzel-Boroviczeny O, Wahle J, Koletzko B. Fatty acid composition of human milk during the 1st month after term and preterm delivery. Eur J Pediatr 1997;156:142-7.
11. Bahrami G, Rahimi Z. Fatty acid composition of human milk in Western Iran. Eur J Clin Nutr 2005;59:494-7.
12. Michaelsen KF, Skafte L, Badsberg JH, Jorgensen M. Variation in macronutrients in human bank milk: Influencing factors and implications for human milk banking. J Pediatr Gastroenterol Nutr 1990;11:229-39.
13. Luukkainen P, Salo MK, Nikkari T. Changes in the fatty acid composition of preterm and term human milk from 1 week to 6 month of lactation. J Pediatr Gastroenterol Nutr 1994;18:355-60.
14. Hornstra G. Essential fatty acids in mothers and their neonates. Am J Clin Nutr 2000;71(5 suppl):1262S-9S.
15. Scopsi F, Ciangherotti S, Lantieri PB, Risso D, Bertini I, Campone F, *et al*. Maternal dietary PUFAs intake and human milk content relationships during the ¢rst month of lactation. Clin Nutr 2001;20:393-7.
16. Nikniaz L Jr, Mahdavi R, Arefhoesseini SR, Sowti Khiabani M. Association between fat content of breast milk and maternal nutritional status and infants' weight in tabriz, iran. Malays J Nutr 2009;15:37-44.
17. Villalpando S, del Prado M. Interrelation among dietary energy and fat intake, maternal body fatness and milk total lipids in humans. J Mammary Gland Biol Neoplasia 1999;4:285-95.
18. World Health Organization. The quantity and quality of breast milk. Report on the WHO Collaborative Study on Breast Milk. Geneva: World Health Organization; 1985.
19. Michaelsen KF, Larsen PS, Thomsen BL, Samuelson G. The Copenhagen Cohort Study on Infant Nutrition and Growth: Breast-milk intake, human milk macronutrient content, and influencing factors. Am J Clin Nutr 1994;59:600-11.
20. Marandi A, Ezzeddin-Zanjani N. On the occasion of the World breast feeding day. The Promotion of Breastfeeding in the Islamic Republic of Iran. Int J Prev Med 2012;3:520-1.

How to cite this article: Kelishadi R, Hadi B, Iranpour R, Darani KK, Mirmoghtadaee P, Farajian S, *et al*. A study on lipid content and fatty acid of breast milk and its association with mother's diet composition. J Res Med Sci 2012;17:824-7.

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