

Fatty Acid Composition in Breast Milk from 4 Regions of Thailand

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Background: DHA contents in breast milk varied upon maternal dietary intakes.

Objective: To study DHA contents in breast milk in Thai lactating women from four different regions of Thailand.

Material and Method: 20 mL of hind milk from 40 lactating women from Bangkok, Chantaburi, Tak, and Surin were collected and analyzed for fatty acids contents by gas chromatography. Dietary intake of lactating women after delivery until the present study was assessed by a food frequency questionnaire. Then, the average DHA intake was estimated from the diets by using the reference data.

Results: DHA contents in breast milk of mothers from Surin were higher than those from other areas. There were no correlations between history of DHA intake and DHA contents in breast milk.

Conclusion: DHA contents in breast milk vary from region to region of the country. Local dietary intake and genetics might explain this contrast.

Keywords: Arachidonic acid, Breast milk, Docosahexaenoic acid, Fatty acids, Thai mothers

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Breast milk has been well accepted to be an ideally role source of nutrients supplied to infants during the first 6 months of life⁽¹⁾. Human milk fat is the major source of energy for infants, providing 40-50% of the total energy intake. It, likewise, provides essential nutrients such as fat-soluble vitamins and n-6 and n-3 polyunsaturated fatty acids (PUFAs) necessary for development of the brain⁽²⁾. The longer chains, the more unsaturated fatty acids synthesized from 18:2n-6 and 18:3n-3 are referred to as long-chain polyunsaturated fatty acids (LCPUFAs). The findings that breastfed infants have higher plasma LCPUFA contents than the infants fed by unsupplemented formulas support the concepts that the amounts of LCPUFA synthesized endogenously are less than those provided by the human milk^(3,4).

Docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6), which are LCPUFAs found in human milk, are major constituents of brain phospholipids^(5,6). DHA accounts only for a small percentage of the fatty acid content in most tissues, except in the retina and the cerebral cortex, and thus is essential for development and functioning of the retina and the brain. Contrary to DHA, ARA is present in relatively large amount in most tissues. It is the major LCPUFA in most peripheral tissues and is present in a large amount in the nervous tissue. It is also the precursor of biological substances known collectively as eicosanoids: prostaglandins, leukotrienes, and thromboxanes, which have the important roles in immunoregulation, inflammatory processes, and muscle contraction.

Fat composition in human milk is influenced by various factors such as maternal diet, duration of pregnancy, and stage of lactation⁽⁷⁾. LCPUFA contents of mature milk are less than those of transitional milk and colostrum⁽⁸⁾. Lactating women who consumed

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more marine fish were shown to have higher DHA and ARA concentrations in their milk⁽⁹⁾.

The authors, thus, studied fatty acid compositions in breast milk and daily DHA consumption at 1 month postpartum of Thai lactating women living in four different regions of Thailand. The results of which will lead to nutritional intervention in some regions for improving LCPUFA status in breast milk of Thai lactating women.

Material and Method

Subjects

One hundred and sixty lactating Thai women who agreed to provide twenty milliliter of breast milk at 1 month after delivery were recruited from four different regions of Thailand. The four regions were Bangkok, Chantaburi, Tak, and Surin. Bangkok is the capital city of Thailand, which is located close to the sea (about 100 kilometres from the sea). Chantaburi, a province in the east region, is located on the sea coast. In contrast, Tak is a province in the northwest area, of which the samples of breast milk are derived from hill-tribe lactating women. Lastly, Surin is a province in the northeast region of the country, which is assumed as the poorest region of the country and quite far away from the sea. Each mother who volunteered to the present study was healthy, and her full-term baby was solely breastfed. A mother who was prohibiting her milk to the baby, had any serious diseases or infection, was on any medications, or was an alcoholic drinker was excluded from the present study. The study protocol was approved by the Ethics Committee for Clinical Research, Faculty of Medicine Siriraj Hospital, Mahidol University. All lactating women who participated in the present study signed their informed consent before being included in the study.

Dietary intakes

Dietary intake of lactating women after delivery until the study period was assessed by a food frequency questionnaire is described elsewhere⁽¹⁰⁾. The weekly frequency of each food intake after delivery was interviewed following the same questionnaire. Then, the average DHA intake was estimated from the diets by using the reference data⁽¹¹⁾.

Breast milk samples

Samples of breast milk were obtained from 40 lactating women in each region. First 10 milliliters of expressed milk was discarded; then, the following 20 milliliters was collected for analysis.

Fatty acid analysis

One hundred microliters of human milk was added to Teflon-lined screw-cap test tubes. Two milliliters of methanol-hexane (4:1, v/v) was added to the milk sample, which was then mixed in vortex vigorously. The amount of 0.2 mL of acetyl chloride was slowly added to the stirring tube over the period of 1 min. The tube was tightly closed with a Teflon-lined cap and subjected to methanolysis at 100°C for 1 hr. After the tube had been cooled at room temperature, 5 mL of 6% K₂CO₃ solution was slowly added to stop a reaction and to neutralize the mixture. The tube was then shaken vigorously and centrifuged at 2900 rpm for 10 min resulting in a separate supernatant containing fatty acid methyl esters, which were analyzed by using a gas chromatography (Trace GC 2000, Thermo Finnigen, Italy)⁽¹²⁾ with a 30 m, 0.32 mmID, 0.25 m fused-silica capillary column (FAMEWAX™, Restek corporation, USA) and a flame ionization detector. Helium was used as a carrier gas at a rate of 25 m/min. The column temperature was programmed to increase from 50°C (hold 1 min) to 100°C at the rate of 10°C/min, then 4°C/min to 200°C with a final hold of 5 min. The detector temperature was 250°C and an injector temperature was 200°C.

The emergent peaks were identified by comparing their retention time with those of the standard fatty acid methyl esters. The relative proportion of fatty acid was derived from the area under each peak divided by the total areas of all fatty acids appeared in the chromatogram. The values were expressed as percentage of total fatty acids⁽¹³⁾.

Statistical analysis

Data were collected and analyzed with the use of SPSS software (version 11.5; SPSS Inc, Chicago, IL). Analysis of variance with multiple comparisons by Tamhane and Bonferroni were used to compare the differences among the four regions. Significant level was set at $p < 0.05$. Pearson correlation was used to assess the correlation of DHA level in breast milk and maternal DHA intake after delivery.

Results

One hundred sixty participants from four representative provinces of the regions participated in the present study. Table 1 shows the average weekly intake of DHA of lactating women living in each province. Thirty-eight, 40, 23, and 39 mothers from Bangkok, Chantaburi, Tak, and Surin completed the questionnaires. Then the DHA contents of their

breast milk were compared with the history of dietary intakes. The lactating women from Surin had a higher DHA intake than those from other provinces. However, there were no correlations between DHA intakes and DHA levels in breast milk among these four provinces ($r=0.22, p=0.01$).

Table 2 shows the fatty acid contents in breast milk of mothers from four provinces. Linoleic acid (LA) contents in milk of mothers from Bangkok are significantly higher than those from Surin ($p=0.001$). α -Linolenic acid (ALA) contents in milk of mothers from Bangkok are significantly higher than those from Chantaburi ($p=0.002$), Tak ($p=0.031$), and Surin ($p=0.014$). Eicosapentaenoic acid (EPA) levels in milk from Bangkok are significantly lower than those from Tak ($p=0.001$) and Surin ($p=0.000$). DHA contents in milk of mothers from Bangkok are significantly lower than those from Chantaburi ($p=0.008$), Tak ($p=0.007$), and Surin ($p=0.000$).

Table 3 demonstrates the DHA contents of breast milk from Bangkok, Chantaburi, Tak, and Surin. DHA contents in milk of mothers from Surin are sig-

nificantly higher than those from other areas of the country ($p < 0.001$).

Discussion

Fatty acid compositions in breast milk vary widely throughout the world^(14,15). It is, thus, difficult to design the ideal fatty acid composition of infant formula. Genetic and environmental factors have played independent roles in affecting breast milk composition. Dietary intakes and types of cooking oil consumed by lactating mothers contribute major impacts on the fat composition in breast milk. Soybean oil, which is high in linoleic acid and linolenic acid, is more expensive than other cooking oils sold in Thailand and is widely consumed by those who are medium-to-high income groups, such as those in Bangkok. On the other hand, palm oil and sunflower oil are cheaper and are consumed more by low-income group. This reflects in significantly higher LA and LNA contents in breast milk of mothers from Bangkok and Chantaburi than those from Surin, which is supposed to be the poorest province in Thailand.

Mean concentrations of LA and ALA in the breast milk from various centers are in the ranges of 14-18% and 0.2-1%, respectively^(8,16,17). The only metabolic significance of higher LA content in breast milk is that it contributes higher allergic symptoms in infants^(18,19).

High marine intake during lactation is a major contributing source of n-3 LCPUFA. From our dietary interview and questionnaires, mothers from Surin consumed the highest marines compared to those from other provinces resulting in significantly higher levels of EPA and DHA in their breast milk. However, there is no significant correlation between the marine intake and n-3 LCPUFA both within the group and among the groups. Other factors such as genetics and other food sources might play the roles in these differences.

Table 1. Weekly DHA intake of lactating women from 4 provinces

	n	DHA intake [□] (mg)	95% confidence interval of mean	
			Lower bound	Upper bound
Bangkok	38	8.12 ± 0.94	6.22	10.02
Chantaburi	40	5.97 ± 0.62	4.72	7.22
Tak	23	1.42 ± 0.20	1.01	1.83
Surin	39	10.44 ± 0.82	8.79	12.10

[□] mean ± SEM

Table 2. Contents of essential fatty acids and LCPUFAs in breast milk from 4 provinces

	Bangkok	Chantaburi	Tak	Surin
LA	17.229 ± 0.738 [□]	16.537 ± 0.593	15.359 ± 0.903	13.143 ± 0.647
ALA	0.559 ± 0.065	0.244 ± 0.031	0.362 ± 0.045	0.424 ± 0.086
ARA	1.046 ± 0.064	1.308 ± 0.227	1.249 ± 0.027	1.281 ± 0.037
EPA	0.077 ± 0.012	0.096 ± 0.008	0.144 ± 0.011	0.279 ± 0.030
DHA	0.397 ± 0.023	0.526 ± 0.031	0.533 ± 0.033	0.783 ± 0.045

[□] mean ± SEM (% of total fatty acids)

Table 3. Comparisons of fatty acid contents in breast milk from 4 provinces

Dependent variables	Site I	Site II	Difference [□]	p-value
LA	Bangkok	Chantaburi	0.960 ± 1.032	NS
		Tak	0.871 ± 1.032	NS
		Surin	4.086 ± 1.032	<0.001
ALA	Bangkok	Chantaburi	0.315 ± 0.085	<0.01
		Tak	0.197 ± 0.085	NS
		Surin	0.136 ± 0.085	NS
ARA	Bangkok	Chantaburi	-0.261 ± 0.068	<0.01
		Tak	-0.203 ± 0.069	<0.05
		Surin	-0.235 ± 0.074	<0.05
EPA	Bangkok	Chantaburi	-0.019 ± 0.015	NS
		Tak	-0.067 ± 0.016	<0.001
		Surin	-0.202 ± 0.032	<0.001
DHA	Bangkok	Chantaburi	-0.128 ± 0.039	<0.01
		Tak	-0.136 ± 0.040	<0.01
		Surin	-0.356 ± 0.050	<0.001

[□] mean ± SEM

DHA concentrations in breast milk from several studies are in the ranges of 0.1 to 0.7% of total fatty acids^(9,20,21), and the differences depend on the maternal intake of marine food sources. In addition, EPA concentrations of maternal milk from Surin are significantly highest among the four studied provinces, which go along with a history of high marine intake of the mothers from Surin. EPA has been shown to reduce inflammation and to improve symptoms of inflammatory skin disease^(22,23). Therefore, higher intake of n-3 LCPUFA in breast milk will prevent allergic symptoms in infants as well as accelerate their development^(24,25). However, the specific roles of LCPUFA in normal development are not clear. Therefore, the differences of LCPUFA concentration in breast milk of women from different regions of the country, as found in the present study, will result in different rates of developments and incidence of allergy in Thai infants.

In contrast to other studies that showed the ARA content in human milk as quite stable, the remarkably low level of ARA in breast milk from mothers in Bangkok should be of concern. The type of cooking oil as well as the small amount of daily marine consumption might play a role in the lowest ARA level in breast milk of women from Bangkok. Blood ARA levels in the

newborn have been connected with growth parameters at birth⁽²⁶⁾, and ARA status in the first weeks after birth is associated with growth up to 1 yr of age in preterm infants⁽²⁷⁾. Thus, the impact of the lowest ARA content in mothers from Bangkok on their infants should be thoroughly studied.

The present study has some limitations. First, data of consumed DHA are derived from retrospective questionnaire. From food frequency questionnaire, the major source of DHA of mothers from Surin was mackerel fish and the authors estimated DHA content by comparing the reference elsewhere⁽¹¹⁾. Such estimation may not be absolutely correct because the consumed fish were not from the same lot of the reference. Second, the authors did not estimate food intake during pregnancy. However, body weight, height, and body mass index of subjects in each group were not different (data not shown); thus, the authors assumed that maternal body stores, a source of fatty acids in human milk, were similar.

In conclusion, the ARA, EPA, and DHA levels of breast milk of medium-income mothers from Bangkok, which is the capital city of the country, are significantly lowest among this study group. Differences in location and in food intake during lactation might have contributed to the differences in fatty acid contents in breast milk. Other factors, such as local food intake, might play an essential role in contributing to high DHA contents in breast milk. However, this remains to be further determined.

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กรดไขมันในนมแม่จาก 4 ภูมิภาคของไทย

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ภูมิหลัง: ปริมาณกรดไขมัน DHA ในนมแม่มีค่าแตกต่างกันขึ้นกับอาหารที่มารดาบริโภค

วัตถุประสงค์: เพื่อเปรียบเทียบปริมาณกรดไขมัน DHA ในนมแม่คนไทยจาก 4 ภูมิภาค

วัสดุและวิธีการ: ผู้ศึกษาได้ทำการสำรวจหญิงให้นมบุตรหลังคลอด 1 เดือน จำนวน 40 คนที่อาศัยอยู่ในจังหวัด กรุงเทพมหานคร จันทบุรี ตาก และสุรินทร์ การศึกษาทำโดยให้หญิงให้นมบุตรตอบแบบสอบถามเกี่ยวกับอาหารที่บริโภคเพื่อนำมาคำนวณหาปริมาณกรดไขมัน DHA โดยคิดเปรียบเทียบกับค่าอ้างอิง นอกจากนี้จะมีการเก็บน้ำนมแม่เพื่อนำมาตรวจปริมาณกรดไขมันด้วยเครื่อง gas chromatography

ผลการศึกษา: กรดไขมัน DHA ในนมแม่ของหญิงให้นมบุตรที่อาศัยอยู่ในจังหวัดสุรินทร์มีค่ามากกว่าหญิงให้นมบุตรที่อาศัยในจังหวัดอื่น แต่ไม่พบความสัมพันธ์อย่างมีนัยสำคัญทางสถิติระหว่างปริมาณกรดไขมัน DHA ในนมแม่และปริมาณ DHA ในอาหารที่มารดาให้นมบุตรบริโภค

สรุป: ปริมาณกรดไขมัน DHA ในนมแม่มีค่าแตกต่างกันในแต่ละภูมิภาค ดังนั้นความแตกต่างในด้านอาหารที่บริโภคและพันธุกรรมอาจเป็นปัจจัยที่ส่งผลต่อระดับกรดไขมัน DHA ในนมแม่
